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Optical Coherence Tomography Angiography versus Fundus Fluorescein Angiography in Assessing Age-Related Macular Degeneration: A Retrospective Study

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Original Research Article

ABSTRACT

Optical coherence tomography angiography (OCTA) could be a valid tool to detect choroidal neovascularization (CNV) in neovascular age-related macular degeneration (nAMD), allowing the analysis of the type, the morphology, and the extension of CNV in most of the cases. This retrospective cross-sectional study, aimed to highlight the role of optical coherence tomography angiography (OCTA) as compared to fluorescein angiography (FA) in the evaluation of age related macular degeneration (AMD).was conducted at tertiary eye care centre. This study enrolled 24 patients (48 eyes). All patients underwent swept-source optical coherence tomography (SS-OCT), swept-source OCTA, and fundus fluorescein angiography (FFA). OCTA was used to evaluate neovascular networks in terms of their type, location and extent of visualization. Sensitivity and specificity of the method were assessed based on FFA diagnosis as the gold standard. In our study, the sensitivity and specificity of OCTA in detecting CNV secondary to wet AMD seem to be high which were 85.1% and 80% respectively. OCT angiography is a clinically useful tool to evaluate the CNV activity and response to treatment as well as to differentiate the various types of CNV in wet AMD.

KEY WORDS: age-related macular degeneration (AMD), choroidal neovascularisation (CNV), flourescein angiography (FA), optical coherence tomography angiography (OCTA)

INTRODUCTION:

Age-related macular degeneration (AMD) is the term applied to ageing changes in macula without any other obvious precipitating cause in people aged 50 years and above[1]. Aging is the strongest risk factor for AMD. Age-related changes in Bruch's membrane and age-related formation of the components of drusen play the strongest role in AMD[2]. AMD has been classified depending on whether there is a presence of abnormal neovascularization into wet (exudative or neovascular) and dry AMD[3].

Neovascular age-related macular degeneration (nAMD), also known as wet age-related macular degeneration, an advanced form of macular degeneration, is the leading cause of visual impairment in older adults related to AMD[4]. The presence of abnormal blood vessels, known as choroidal neovascularization (CNV), can penetrate Bruch's membrane (BM) and extend into the subretinal pigment epithelial (RPE) or subretinal space. CNV can induce haemorrhage, fluid exudation, and fibrosis, resulting in photoreceptor damage and vision loss[5]. Aging is the strongest risk factor for AMD, but is not modifiable. Early visualization and diagnosis of the CNV lesion are essential to prevent progressive, irreversible vision loss.

As the current gold standard of determining the presence of leakage on fluorescein angiography (FA) can provide dynamic information[6]. In the late phase of the angiogram leakage of dye is used to diagnose and classify CNV as classic, occult, or combination subtype. However, it is an invasive procedure, requiring intravenous dye injection, which can induce nausea, discomfort, and occasionally anaphylaxis[7,8]. In addition, this technique is time consuming, taking about 15–20min to complete, which can limit its routine use in a busy clinical setting.

For these reasons, optical coherence tomography (OCT) was introduced. OCT has become a widely used non-invasive imaging technique these days to detect the presence and activity of CNV without the use of intravenous dye. It enables

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visualization of the morphological features of the fibrovascular complex and the exudative consequences of fluid accumulation, which is accompanied by retinal thickening and edema[9]. However, sensitivity of OCT is only to the backscattering light intensity and can't distinguish vasculature from fibrous and other surrounding tissues. Because of this limitation, it is very difficult to recognize the the precise location and activity of the CNV. Thus, OCT imaging cannot replace but supplement FA in the diagnosis of nAMD[10].

Optical coherence tomography angiography (OCTA) is a novel imaging modality that allows direct visualization of the retinal and choroidal vasculature in vivo. In OCTA, high-frequency and dense volumetric scanning are applied to detect blood flow by analysing the signal decorrelation between scans. Compared with stationary areas of the retina, the movement of erythrocytes within a vessel generates a decorrelated signal[11]. Unlike traditional angiography, OCTA does not require the use of exogenous dyes, thus avoiding potential side effects, such as nausea or other more serious adverse events.

However, the role of OCTA in diagnosing neovascular age-related macular degeneration has not been widely investigated. Very few clinical studies [12] have evaluated the accuracy of OCTA imaging for the diagnosis of nAMD. Therefore, this study was conducted to evaluate the efficacy of OCTA in detecting nAMD.

MATERIAL AND METHODS:

A retrospective cross-sectional review based analysis of data was done at Retina Institute of Karnataka, Bangalore during usual clinical practice from consecutive patients diagnosed with nAMD. Clinical and instrumental assessments were performed in 24 patients (48 eyes). The subjects included in this study were patients over 50 years with clinical features of age-related maculopathy, such as soft or hard drusen, pigmentary alterations and macular exudative signs on FFA.

All the patients underwent a comprehensive eye examination, which included slit lamp biomicroscopy, colour fundus photography, swept source OCT (SS-OCT) and swept source OCTA (SS-OCTA) using Topcon OCT Triton. The angioretina of the Topcon OCT Triton utilizes OCTARA algorithm. OCTA acquisition protocol in the macular region consisted of a 6×6 mm area centred onto the fovea. En face OCTA images were segmented into four layers, namely the superficial vascular plexus, deep vascular plexus, outer retina, and choriocapillaries. Exclusion criteria were (a) patients without OCTA or FA results available for analysis or ,patients with CNV secondary to pathological myopia, angioid streaks, chorioretinitis, central serous chorioretinopathy, tumors, or trauma; (b) media opacities, such as cataracts, (c) preventing detailed imaging, history of posterior segment surgery within the last 6 months, history of laser photocoagulation and (d) any contraindication to intravenous fluorescein injection as renal impairment and hypersensitivity.

For FA, according to the criteria of the Macular Photocoagulation Study[13,14], the CNV lesions were graded as classic, occult, and combination. Classic CNV was defined as an area of uniform and early (<30 sec) hyperfluorescence leakage throughout the middle and late phases. Occult CNV was identified by fibrovascular pigment epithelial detachment (stippled hyperfluorescence) or latephase leakage of an undetermined source. The appearance of CNV on the OCTA images and coregistered corresponding OCT B-scans was assessed, in addition to the presence of subretinal fluid, intraretinal fluid, or sub-RPE fluid. CNV was defined as the presence of a decorrelation signal at the outer-retina level on OCTA consistent with the vascular component of the lesion. The appearance of CNV on an OCTA image was classified as either well circumscribed (lacy wheel or sea fan-shaped vessels) or poorly circumscribed (long filamentous vessels).

All statistical interpretation and analysis of results obtained were carried out using statistical software SPSS version 22 (Statistical Package for Social Sciences, version 22, SPSS Inc, Chicago, IL, USA) and Microsoft Excel.

RESULTS:

We reviewed 24 patients (48 eyes) with macular degeneration who visited the Institute. Eleven eyes were excluded because of poor quality images attributable to poor fixation, media opacity, absence of OCTA or FA results. 37 eyes of 24 patients were assessed. The patients consisted of 15 men and 9 women aged between 50 and 85 years with mean age, 67 years (Table 1). 27 eyes were diagnosed as having CNV with FA, with 3 patients diagnosed as having bilateral nAMD. According to FA, the CNV lesions were classified as classic in 14 eyes, occult in 11 eyes. And mixed in 2 eyes.

The qualitative tomographic OCTA review showed signs of CNV in 23 eyes. Occult CNV lesions (type I on OCT) were best visualized on the SS-OCTA

Table 1: Demographic and clinical data of the study patients.

Gender		
	Male	15
	Female	9
Age		
	Mean	67
	SD	10.3
	Min	50
	Maximum	85
AMD Classification		
	Classic	14
	Occult	11
	Mixed	2

Table 2: Detection of eyes with neovascular age-related macular degeneration using optical coherence tomography angiography compared to fluorescein angiography.

	Fluorescein Angiography		
OCTA	Positive	Negative	Total
Positive	23	2	25
Negative	4	8	12
Total	27	10	37

Table No.3 Sensitivity, specificity, and predictive value of SSOCTA in detecting neovascular age related macular degeneration.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
OCTA	85.1	80	92	66.6

en face projection of the choriocapillaris, whereas classic CNV lesions (type II on OCT) were best visualized on the SS-OCTA en face projection of the outer retina. Mixed CNV lesions can be seenon the SS-OCTA en face projection of both the outer retinaand choriocapillaries layer (Figure 1). OCTA enabled accurate localization of neovascular networks with respect to the RPE layer. 2 false-positive cases were observed on OCTA (Figure 2), (Table 2). There were 4 false-negative cases (Figure 3). The specificity of OCTA for the detection of CNV was 80%, with a sensitivity of 85.1% and positive and negative predictive values of 92% and 66.6%, respectively (Table 3).

DISCUSSION:

FA can detect dynamic patterns of dye transit and leakage and keeps the current gold standard for diagnosing CNV[6]. However, traditional angiography is invasive and time consuming. Other major limitations are that it provides only a two-dimensional image and cannot directly visualize nascentvessels. SD-OCT is increasingly used in clinical practice to determine both the presence and activity of CNV. It can not replace FA as the gold standard in the diagnosis of nAMD, because the reflectivity of CNV tissue and drusenoid material, hemorrhages, and RPE are similar on OCT. Therefore, it is highly desirable to develop a novel method, such as OCTA, for monitoring nAMD. OCTA can simultaneously provide functional (OCT angiograms) and morphological (OCT B-scans) information and may be performed monthly because it is simple, quick, and non-invasive[15].

Current study aimed to assess the ability of the OCTA technique in detecting active CNV and to determine efficacy (sensitivity and specificity) of SS-OCTA. In our study OCTA has proved reliable in distinguishing between the two types of CNV: occult and classic, new blood vessels growing beneath or above the RPE, respectively.

During study we found a dark halo surrounding active CNV lesions and that was displayed as a hypointense clear zone on SS-OCTA images. This finding is consistent with Jiaet al[16] and Coscaset al.[17] who reported the same sign. An explanation for this dark halo is that CNV tends to develop a region of choriocapillaris alteration caused by impaired flow to compensate for ischemia. The region of choriocapillaris alteration is located underneath the CNV and extends beyond its margins in the form of a ring or halo and appears as hypointense or silent area on SS-OCTA images due to reduced blood flow[18].

In our study the sensitivity and specificity of OCTA in detecting the CNV secondary to nAMD was 85.1% and 80% respectively, almost same as that of Faridi A et al[19]. Four false negative eye with no decorrelation signal on en face OCTA due to sub-retinal haemorrhage. This finding is consistent with other studies, which have reported a decreased ability of OCTA to detect CNV in eyes with subretinal-haemorrhage. Moult et al[20] reported in their series that the single case in which OCTA revealed false-negative result had dense subretinalhemorrhage that caused severe attenuation of the SS-OCT signal. Faridiet al[19] concluded that sensitivity of en face

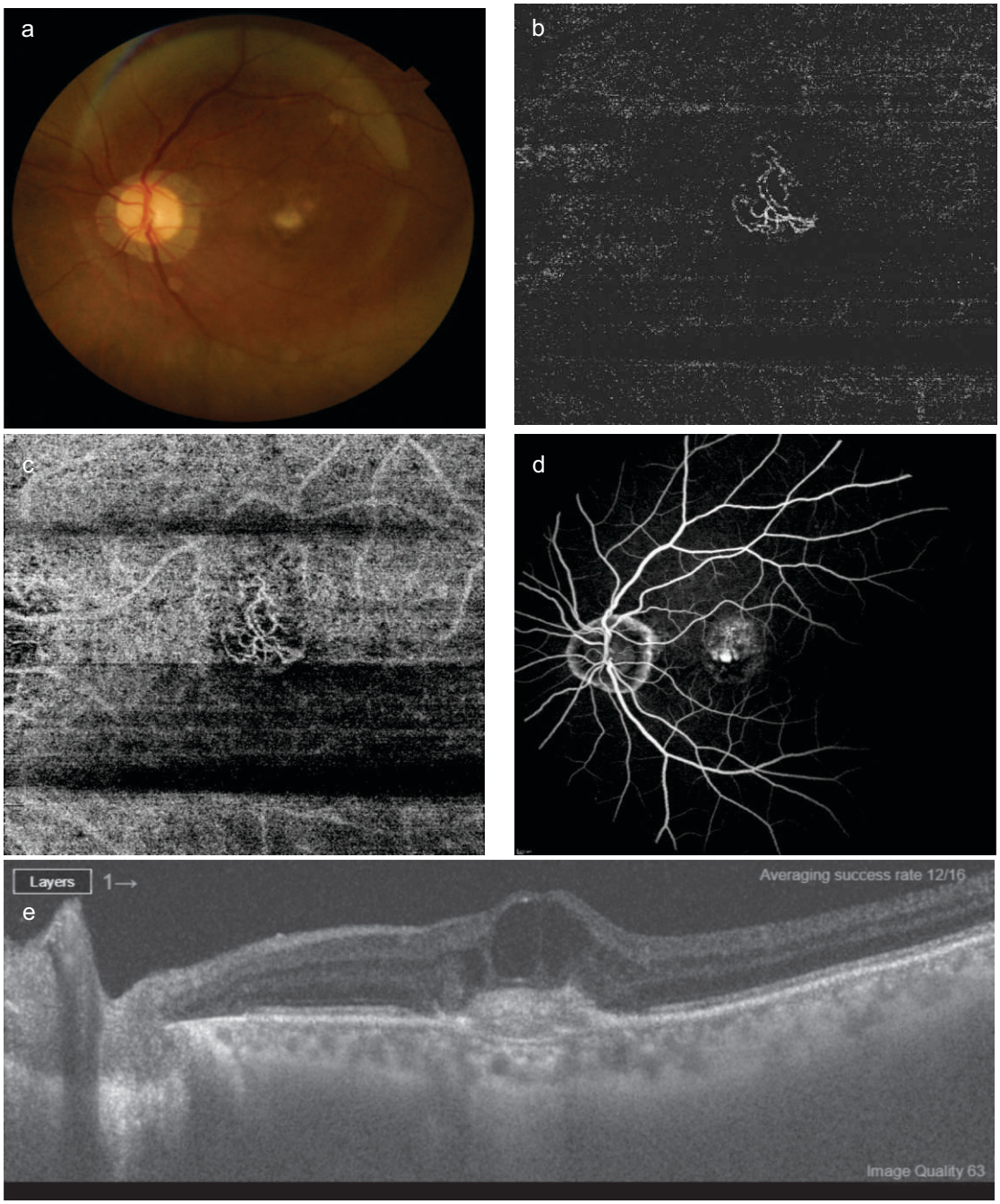

Figure 1.(Original)Multimodal imaging of a type II choroidal neovascularization (CNV), evaluated as a true positive case (a)Fundus of left eye showing yellow lesion at fovea .(b-c) A 6 × 6mm optical coherence tomography angiography slab at the outer retina and at the choriocapillaris showing a well-circumscribed branched CNV. (d) late frame fluorescein angiography displaying a small area of late leakage (white arrow) in the foveal area (e) OCT showing cystoid spaces and subretinal hyper-reflective material.

OCTA improved to 94% if eyes with subretinalhemorrhage were excluded. Jiaet al[16] demonstrated the ability of OCTA to detect and quantify CNV in 10 patients with wet AMD where OCTA provided better visualization of the neovascular network with respect to FA, as images were not obscured by subretinalhemorrhage or other artifacts. De Carlo et al[12] reported that the sensitivity and specificity of OCTA in detecting CNV secondary to wet AMD were 50% and 91%, respectively. Low sensitivity was due to small sample size and blockage from large amounts of retinal hemorrhage in some patients. Nikolopoulouet al[21] reported that the sensitivity and specificity of OCTA in detecting CNV

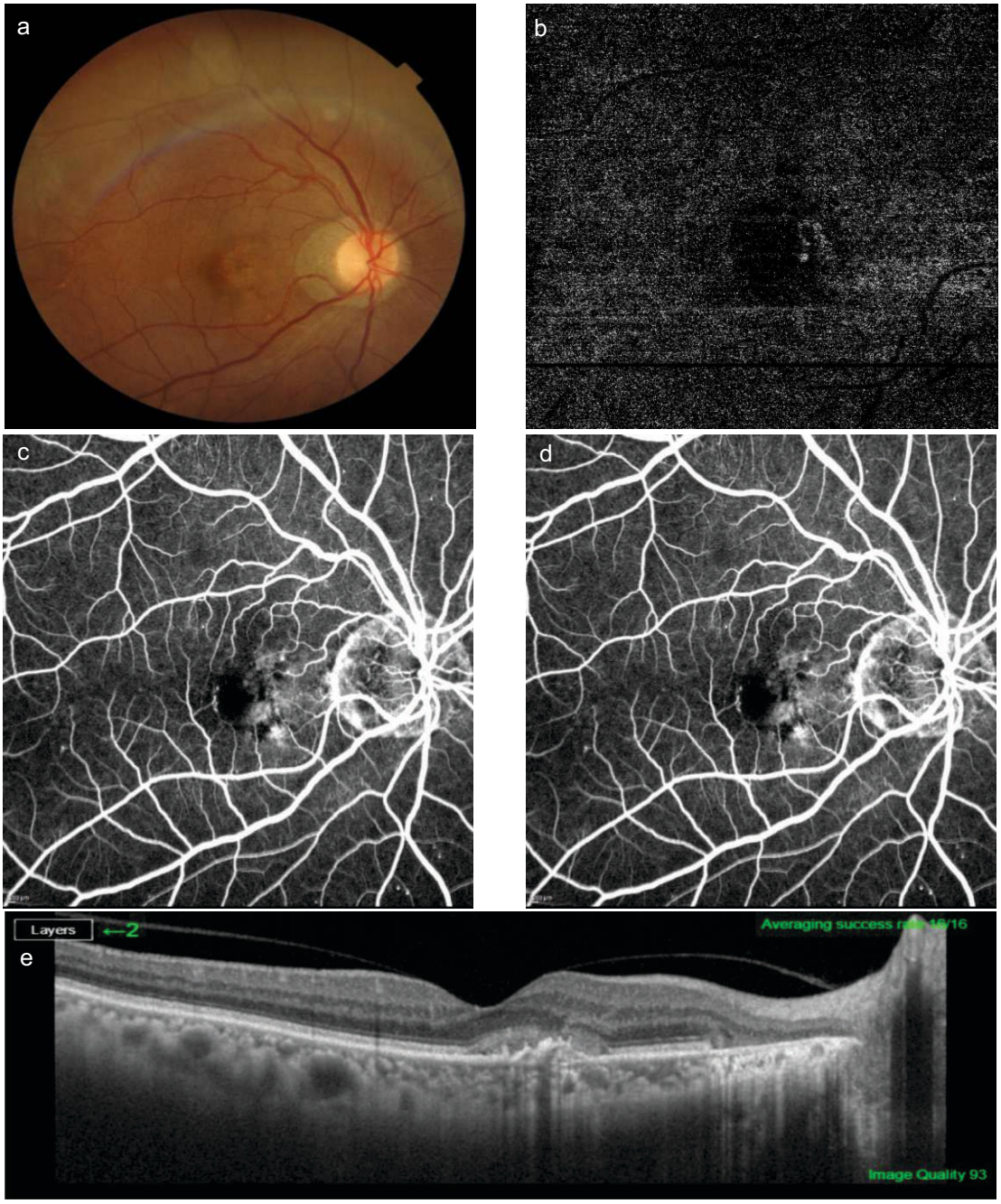

Figure 2: (Original)Images of choroidal neovascularization (CNV) observed in false-positive case on optical coherence tomography angiography (OCTA). (a) Fundus photo from a 62 -year-old man showing mild RPE irregularity (b) A 6∗6-mm En face optical coherence tomography angiogram of the outer retina showing a CNV in the juxtafoveal space (white arrow). (c and d) Early- and late-frame FA images of the patient displaying mild hyperfluorescence that is stable throughout the FA in the region of CNV without pooling. (e) Mild RPE irregularity seen on the spectral-domain optical coherence tomography corresponding to fundus picture.

secondary to wet AMD 88% & 90 % respectively.

In our study OCTA showed high sentivity in detecting type I CNV. This result was partially in discordance with Nikolopoulouet al[21] who stated that OCTA would display worse sensitivity in naïve CNV, due to undetectable flow inside the small peripheral branches of the neovascular complex.

In our study active CNV on OCTA showed well-defined complexes, dark halo around the lesion and numerous tiny anastomotic capillaries with thin walls and small diameter. There was excellent level of correlation in treatment decision based on OCTA compared to FFA. This result is consistent with findings of Coscaset al[17] and Spaideet al[22]. Coscaset

Figure 3: (Original)Images of subretinalhemorrhage observed in false-negative case on optical coherence tomography angiography (OCTA). (a)Color fundus photograph of the patient subretinalhemorrhage. (b) A 6∗6-mm En face angiogram of choriocapillaries not showing any CNV (c) Late-frame FA image displaying big leakage and pooling at the around the macula together with posterior pole pigment epithelium detachment. (d) Spectral-domain optical coherence tomography (SD-OCT) demonstrating subretinalhemorrhage with the retinal pigment epithelial (RPE) detachment.

al[17] had compared the OCTA with traditional multimodal imaging in patients with wet AMD and found that there was high inter-observer agreement both for treatment decision in conventional multimodal and for pattern I (active CV) or pattern II (inactive CNV) definition in OCTA imaging analysis.

SS-OCT can provide clues suggesting the presence of CNV, such as the presence of SRF, IRF, and subretinal hyper-reflective material, and allows identification of areas where the RPE is separated from BM.

In our study, the sensitivity of OCT along with en face OCTA in detecting the active CNV secondary to AMD compared to FFA was 85.1% and specificity was 80%. Our findings demonstrate that OCTA is able to detect the neovascular complex in most of the cases

of nAMD, allowing the analysis of the morphology of the CNV in every single patient.

CONCLUSION:

OCT angiography is a clinically useful tool to evaluate the CNV activity and response to treatment as well as to differentiate the various types of CNV in wet AMD.

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Causality of Hematology Sample Rejection: A Training Needs Assessment for Health Facilities

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Department of Pathology, NKP Salve Institute of Medical Sciences & Research Centre, Nagpur

Original Research Article

ABSTRACT

A cross sectional study was conducted to assess pre-analytical variables and sources of errors observed among hematology specimen received from IPD area in Central Pathology Laboratory of NKP Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra during July 2016 - June 2017. Rate of sample rejection was noted to be highest from Orthopedics followed by Obstetrics and Gynecology, Medicine, Pediatric, Surgery, Eye, ENT, Pulmonary & Dermatology. The commonest cause for rejection was clotted samples. Other causes were hemolyzed sample, inadequate sample, excessive delay in sample transport and wrong patient identification. Faulty phlebotomy techniques, inappropriate preservation, improper transportation, wrong patient identification, lesser efficiency and carelessness were identified as other reasons.

KEY WORDS: hematology, phlebotomy, rejection, training needs assessment (TNA)

INTRODUCTION:

The clinical diagnosis is largely dependent, these days, on reliable laboratory data. Hence, there is major improvement in the laboratory performances augmented by advances in sample collection, transport, automation and dispatch of reports[1]. Medical laboratories play vital role in the decision making by physicians. About 60-70% of clinical decisions regarding admission, prescription, and discharge are based on laboratory results. Since these play a significant role, the quality of laboratory test results are important[2]. However, errors can occur in any phase during the processing of blood sample. The errors in laboratory practice are classified into pre-analytical, analytical, and post analytical phase depending on the time of presentation[3,5]. An important component of laboratory medicine is pre-analytical phase[5]. The pre-analytical phase comprises of all the processes occurring before the sample being actually processed in the laboratory[6]. It includes specimen collection, handling and processing variables, physiological variables, and endogenous variables. Certain pre-analytical variables, namely, specimen variables can be controlled; whereas knowledge of uncontrollable variables needs to be well understood in order to separate their effects from disease related changes affecting laboratory results[4,7]. The reported types of pre-analytical error are ordering tests on the wrong patient, misidentifying the patient, ordering the wrong test, missing sample and/or test request, wrong or missing identification, contamination from infusion route, hemolyzed, clotted, and insufficient samples, inappropriate containers, improper labeling of containers, inappropriate blood to anticoagulant ratio, and inappropriate transport and storage conditions[8,9]. The laboratories have to bear the burden of the inconsistencies or incorrect reporting that is because of these pre-analytical errors[6]. Analytical errors can be minimized with the recent advancement in technology and introduction of automation in hematology laboratories provided good quality control practices are followed[10]. In spite of automation in hematology and clinical pathology, there are many variables which can influence the laboratory results[11].

The Central Pathology Laboratory (CPL) is routinely functional for 24 hours throughout the year. Properly collected blood sample is essential for quality performance by the Laboratory. Hematology testing is performed on whole blood. Hence, the laboratory data & reliability entirely depends on submitted samples if they are adequate, labeled, and properly transported to the laboratory with in time as per the required protocol. Therefore, the present study was undertaken to assess

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Table 1 : Rejected Specimen Ratio With Respective Department.

SNO	MONTH	REJECT	MED	OBGY	SURG	OPTH	ORTH	ENT	PAED	PSY	TB	SKIN
1	Jul	85	2	20	21	4	15	6	12	3	2	6
2	Aug	85	3	21	22	5	16	6	11	2	1	4
3	Sep	78	3	16	18	4	15	6	13	2	1	6
4	Oct	88	1	14	18	6	13	3	14	2	0	2
5	Nov	72	2	15	20	4	14	5	12	2	2	3
6	Dec	65	1	13	16	4	15	6	14	2	1	8
7	Jan	71	1	16	18	5	14	6	10	2	0	6
8	Feb	72	1	17	20	4	15	5	9	1	1	4
9	Mar	78	1	18	20	6	13	3	8	2	2	8
10	Apr	80	2	16	18	4	14	2	12	4	2	8
11	May	84	2	18	26	6	15	6	12	3	2	10
12	Jun	98	3	19	23	4	16	4	16	3	2	10
13	TOTAL Rejection	956	22	203	240	56	175	58	143	28	16	75
14	REJECT (%)	1.75	2.43	3.02	2.2	1.03	3.6	1	2.6	0.08	0.6	0.26
15	Total recived sample	30153	915	6745	5692	1843	3826	2092	4973	1278	498	2291

* Total recived sample by Randomize collection i.e. 10% of total aggregate sample.

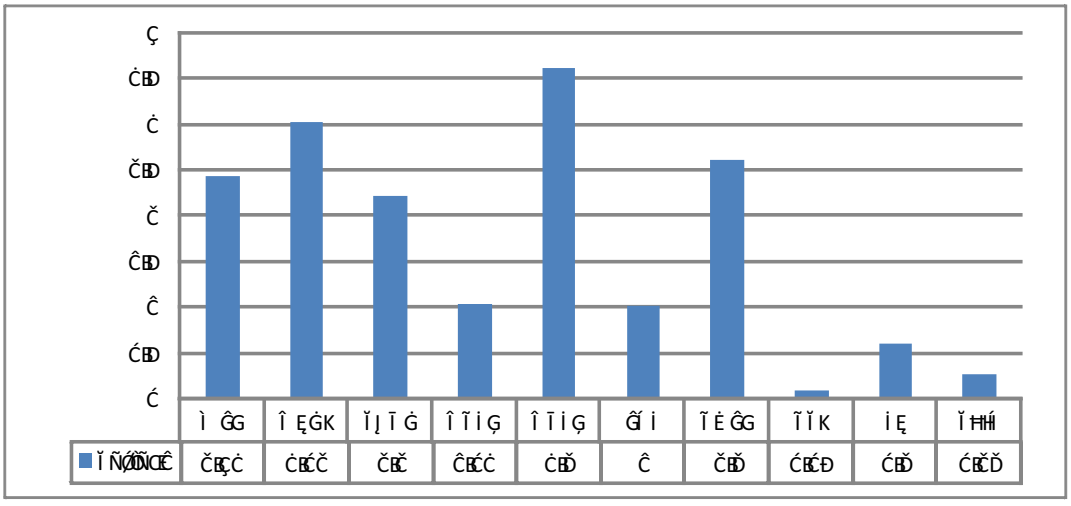

Figure 1: Rejected Specimen Ratio With Respective Department.

prevalence and types of error at CPL in hematology section of IPD patient[12].

MATERIAL AND METHODS:

A cross sectional study was carried out in hematology section of NKP Salve Institute of Medical Sciences and Research Centre, and Lata Mangeshkar Hospital, Nagpur during July 2016 to June 2017.

The data was retrieved from laboratory records of IPD register. Specimen rejection criteria as per SOP protocol included: (a) improperly filled forms; (b) improperly labeled samples (i.e. incorrect label, unlabelled specimen, lost sample); (c) inadequate quantity of sample (i.e. under filled);(d) clotted sample; (e) spillover of sample (i.e. insufficient quantity); (f) hemolyzed sample;

Table 2 : Rejected Specimen Ratio With Respective Reasons.

SN	REJECTION CRITERIA	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
1	Improperly filled forms (1.98%)	0	2	2	1	2	3	1	0	3	3	0	2
2	Improperly labeled samples (1.88%)(i.e. incorred lable, unlabelled specimen, Lost sample)	1	1	0	2	1	2	2	0	1	6	0	2
3	Inadequate quantity of sample (3.66%) (i.e. Under filled)	2	3	2	4	3	3	2	10	2	7	0	0
4	Clotted sample (77.92 %)	78	74	70	56	45	38	51	52	70	60	79	72
5	Spillover of sample (2.92%) (i.e.insufficient quantity)	1	1	0	5	0	5	4	4	1	2	2	3
6	Hemolysed sample (8.26 %)	3	3	4	15	13	8	8	2	1	0	3	19
7	Sample received after 4hrs of collection (0%)(i.e. Too old to process)	0	0	0	0	0	0	0	0	0	0	0	0
8	Inappropriate vacute (0.1 %) (i.e. incorrect patient, incorrect specimen, contaminated specimen, Broken and leaking specimen)	0	1	0	0	0	0	0	0	0	0	0	0
9	Excessive (i.e. Over filled) (2.71 %)	0	0	0	4	8	6	3	2	0	2	0	1
	Total - 956	85	85	78	87	72	65	71	70	78	80	84	98

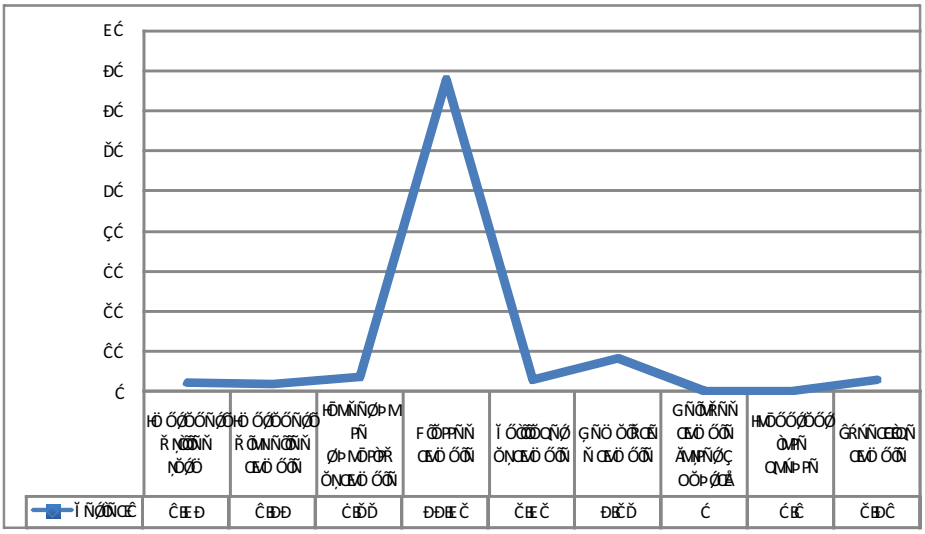

Figure 2: Rejected Specimen Ratio with Respective Reasons.

(g) sample received after 4 hrs of collection (i.e. Too old to process); (h) inappropriate vacute (i.e. incorrect patient, incorrect specimen, contaminated specimen, Broken and leaking Specimen); and, (i) excessive (i.e. over filled).

The areas of collection as well as the reason of rejection were recorded and the results were calculated.

RESULTS:

Although total number of IPD samples received during the study period were 358930, the

study could be held in 30153 samples (8.4%) due to limitations of time and other resources, out of which 956 (3.17% of the samples under study) were received as rejected samples. The contributing areas and reasons of rejection were recorded. Overall rejection rate was observed in the range of 0.26% - 3.6% (Table 1). Highest rejections were seen from Orthopedicward (3.6%), followed by Obstetrics and Gynecology (3.02%), Medicine (2.43%), Pediatrics (2.6%), Surgery (2.2%), Ophthalmology (1.03%) and Otorhinolaryngology (1.0%). Lowest rejection rate is observed in Pulmonary Medicine (0.6%) and Dermatology (0.26%). Commonest cause of rejection was clotted sample (77.92%). Other causes of rejection included hemolyzed sample (8.26%), inadequate quantity (3.66%), spill over of sample (2.92%), excessive quantity (2.71%), inappropriately labeled sample (1.88%), inappropriate vacute (0.1%) and inappropriately filled forms (0.1%) (Table 2).

DISCUSSION:

Modern methods have been applied in medical laboratory to reduce the errors at pre-analytical, analytical, and post analytical phases of sample processing[13]. However, these are commonly found in pre and post analytical phases than that in analytical phase, being so mostly due to factors beyond control of laboratory personnel[14]. Pre-analytical errors are largely being caused by human mistakes and majority of these errors are preventable,[8,15,16] since the pre-analytical phase involves much more human handling compared to the analytical and post analytical phases[17]. Use of automated analyzers in analytical phase has helped to minimize the laboratory errors. Introduction of automated robotic workstations at pre-analytical stage reduces hazards and errors[13] as evidenced by automation of steps and reduction of manual steps involving more people ,bar-coding, simplify specimen routing and tracking[18]. Computerized order simplifies test ordering and eliminates transcriptions errors. Automated phlebotomy tray preparation provides a complete set of labeled blood tubes and labels for hand labeling in a single tray for each patient.

The present study infers that pre-analytical errors were common in IPD samples presumably due to (a) varied urgencies and requirements of hospitalized patients, and (b) spectrum of sample collectors and transport mechanisms[2]. Nurses and paramedical staff collected the samples in IPD and many of those hence need to emphasize on strict observance of SOP related to sample rejection criteria. Hence, capacity building of nurses, paramedical staff & non technical staff requires continued training and guidance for blood sample collection and other interventions. Most common error in this study was clotted samples (8.25%). The presence of clots in EDTA samples can be explained primarily due to increased blood to additive ratio (inadequate EDTA) or improper mixing of the sample after collection[10,17]. In our study, clotted samples could be due to improper mixing. Hemolysis of samples, noted to be 0.03% in this study, occurs when (a) blood is forced through a needle, (b) tubes are shaken vigorously, and (c) sample is centrifuged before clot formation[19]. It results in higher turn-around time since fresh samples require additional time for processing. Detection of hemolyzed samples is relatively difficult in hematology laboratories than biochemistry laboratories and hence may have lower frequency of pre-analytical errors[6]. Inadequate sample size (3.66%) could be due to ignorance of phlebotomists, difficult sampling in pediatrics, patients with chronic debilitating diseases, and patients on chemotherapy with thin veins[6]. Less than recommended volume of withdrawn blood containing ethylene-diamine-tetra-acetic acid (EDTA) results in risk of cell shrinkage and low mean corpuscular volume[20]. Other rejected samples are herein categorized as spill-over samples (2.92%), excessive (over-filled 2.71%), improperly filled (1.98%), improperly labeled sample (incorrectly label, unlabelled & lost samples: 1.88%).

Pre-analytical errors in hematology laboratory (here,1.75%) identifies specimen collection as its commonest cause (Table 2)[6,10,21]. It can however be reduced by competency check of staffs through practical and theory assessment at regular intervals and attending continuing technical education programs especially quality control in hematology.

CONCLUSION:

Looking into the identified intervention areas predominantly amongst those being skill enhancement trainings and re-trainings of all stakeholders including but not limited to nursing and paramedical staff, the service delivery through methodical, appropriate and patient friendly approach. It can be done through better coordination between labs and wards, continuing technical support to laboratory staff, computerization, laboratory information system and proficiency test of staffs. Such capacity building drive shall help in minimizing errors

of sample collection and transport to hematology laboratory.

LIMITATIONS OF THE STUDY:

1. Duty errors of various shifts (viz. those related to evening shift, night shift and holidays etc) and comparative study of different testing centers/ test levels were not included in the study protocol.
2. Time between sample collection and actual analysis was not calculated.

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Doppler Echocardiographic Evaluation in Obstructive Sleep Apnoea

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Original Research Article

ABSTRACT

Obstructive sleep apnoea (OSA) is the commonest form of sleep disordered breathing. Cardiovascular morbidity and mortality are serious complications of OSA. This study was done to evaluate cardiac parameters by Doppler Echocardiography (DE) in patients with OSA in central India. Fifty patients with OSA, diagnosed on full night polysomnography, were recruited into the study. DE was done following standard guidelines. Eighty eight percent of the patients with OSA had normal DE. Thirty one patients were hypertensive. A mild increase in PAP was noted in 6 (12%) patients. Mild LV dysfunction was present in 4 patients. Six patients had diastolic dysfunction. Abnormal DE parameters were present in patients with severe OSA only. A binary logistic regression analysis showed that DE changes were seen in OSA patients with hypertension. Majority of the patients with OSA of all severity had normal DE. Severe OSA, in some cases, was associated with abnormal DE. Abnormal DE in these patients was related to hypertension than to OSA.

KEY WORDS: diastolic dysfunction (DD), doppler echocardiography (DE), left ventricle ejection fraction (LVEF), obstructive sleep apnoea (OSA), pulmonary artery pressure (PAP)

INTRODUCTION:

Obstructive Sleep Apnea (OSA) is the most common form of sleep disordered breathing.[1] OSA may cause many complications and morbidities.[2] Cardiovascular disturbances are one of the serious complications of OSA. These complications include hypertension, coronary artery disease, cardiac arrhythmias, corpulmonale and sudden nocturnal death. Pulmonary hypertension (PH) leading to corpulmonale is a potential complication of OSA.[2]

Doppler Echocardiography (DE) is a modern non-invasive technique to assess the cardiac status. Hence in the present study, we evaluated OSA through DE.

MATERIAL AND METHODS:

In a hospital based, prospective, non-randomized, cross sectional study done over a period of one and half years from January 2017 to June 2018 we evaluated 50 patients of OSA with DE. The study was approved by Research Advisory Committee and Institutional Ethics Committee of People's College of Medical Sciences & Research Centre, Bhopal and All India Institute of Medical Sciences (AIIMS), Bhopal.

Screening for OSA : Patients between the age of 18-80 years were enrolled in the study. Those with sleep complaints like loud snoring, choking, increased day time sleepiness and other risk factors were evaluated for OSA. These patients were subjected to complete physical examination, assessment of Mallampati score and Epworth Sleepiness Score (ESS). Neck circumference (NC) was measured. NC of $\geq$17 inches in men and $\geq$16 inches in women was considered high risk for OSA. Oral examination was done to rule out macroglossia and tonsilar hypertrophy.

Demographic variables including age, gender and occupation were recorded and other clinical variables including breathlessness, hypertension, diabetes mellitus, Body Mass Index (BMI), Electrocardiogram (ECG) changes, Chest X ray and Thyroid profile were also obtained.

Polysomnography: The screened patients were subjected to full night polysomnography (using Philips Alice-6 Diagnostic System) for confirmation of OSA.

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Table 1: Demographic data and Baseline characteristics of study population (n=50).

Baseline variables		Mild OSA (AHI = 5-15/hour)	Moderate OSA (AHI>15 -30/hour)	Severe OSA (AHI>30/hour)	Total
Age (in years)	25 -35	0	0	2	2
	35-45	0	2	4	6
	45 -55	1	2	14	17
	55-65	1	2	18	21
	65-75	0	0	4	4
Gender	Male	1	4	29	34
	Female	1	3	12	16
BMI (in Kg/m^2)	<18.5	0	0	1	1
	18.5 -24.9	1	1	4	6
	25.29.9	0	3	8	11
	30 -34.9	0	0	14	14
	35 -39.9	1	1	7	9
	>40	0	1	8	9
WC (in cm)	Male (>90)	0	0	0	0
	Female (>80)	0	0	0	0
WHR	Male (=0.9)	1	4	28	33
	Female (=0.85)	1	2	12	15
NC (in cm)	Male (>17)	0	1	9	10
	Female (>16)	0	0	1	1
Mallampati score	1	0	0	5	5
	2	0	3	1	4
	3	1	0	11	12
	4	1	3	25	29
ESS	ESS <10	2	3	26	31
	ESS>=10	0	3	16	19
Hypertension	BP≥140/90mmHg	2	4	25	31

(AHI= Apnea Hypopnea Index; BMI=Body Mass Index, WC=Waist Circumference, WHR=Waist Hip Ratio, NC= Neck circumference, ESS=Epworth Sleepiness Score)

Doppler Echocardiography : DE (using Siemens Acuson X300) was performed in patients with confirmed diagnosis of OSA. More than 3 cardiac cycles were used to measure DE parameters. Left ventricular posterior wall thickness (LVPW) was measured using M mode in parasternal long axis view, Left Ventricle Ejection Fraction (LVEF) was calculated by the modified biplane Simpson method. Mean Pulmonary Artery Pressure (PAP) was estimated using Tricuspid Regurgitation (TR) jet velocity.

Statistical analysis : All the data analysis was done using SPSS (Statistical Package for the Social Sciences) ver. 20. Quantitative data is expressed as mean ± SD whereas categorical data is expressed as number and percentage. Student-t test and one way ANOVA was used for quantitative data whereas chi Square test was used for categorical data. Level of significance was assessed at 5%.

RESULTS:

Fifty patients with polysomnography confirmed diagnosis of OSA were selected for DE evaluation. There were 2 patients with mild OSA, 6 with moderate OSA and 42 with severe OSA. General characteristics of patients and DE variables are depicted here in (Table 1 & Table 2).

Majority of the patients (n=46) with OSA had normal LVEF of >50%. Four patients had mildly deranged LVEF of 40-49%. All these 4 patients had severe OSA.

Maximum number of patients ie, 44 (88%) had no Diastolic Dysfunction (DD). Grade 2 DD was

Table 2: DE characteristics of study population (n=50).

	DE variables	Mild OSA	Moderate OSA	Severe OSA	Total	p value
LVEF (%)	<29% (Severe dysfunction)	0	0	0	0	NA
	≥30- 39% (Moderate dysfunction)	0	0	0	0	NA
	40-49% (Mild dysfunction)	0	0	4	4	0.046
	>50% (Normal)	2	6	38	46	0.031
DD (Grades)	0	2	6	36	44	-
	1	0	0	0	0	NA
	2	0	0	5	5	0.432
	3	0	0	1	1	0.876
TR	Present	0	0	0	14	0.021
	Absent	2	6	28	36	-
PAP (mm Hg)	15-22 (Normal)	2	6	36	44	0.041
	>22-40 (Mild PAH)	0	0	6	6	0.048
	41-55 (Moderate PAH)	0	0	0	0	NA
	>55 (Severe PAH)	0	0	0	0	NA

(LVEF= Left Ventricular Ejection Fraction, DD= Diastolic dysfunction, TR= Tricuspid Regurgitation, PAP= Pulmonary Artery Pressure)

present in 5 (10%) patients. Grade 3 DD was present in only one patient. No patient had grade 1 DD. All patients with Grade 2 and 3 DD had severe OSA. Tricuspid regurgitation (TR) was present in 14 (28%) patients (Table 2). TR was present only in patients with severe OSA. Almost all OSA patients (n=44) had normal Pulmonary Artery Pressure (PAP). Mild rise in PAP (>22-40 mmHg) was present in 6 patients. All of them had severe OSA.

Binary logistic regression analysis showed a positive correlation between cardiac parameters like TR, DD, LVEF and PAP and presence of hypertension in patients with OSA. There was a higher OR for the presence of TR, high RVSP, DD, and reduced LVEF in hypertensives with OSA than in non-hypertensives with OSA (Table 3).

Table 3: Binary logistic regression analysis of cardiac parameters with hypertension and OSA (adjusted OR).

DE Variables	OSA patients with Hypertension	OSA patients without Hypertension
TR (present)	OR=1.21, p=0.032	OR=0.11, p=0.342
DD (grade 2 and 3)	OR =1.57, p=0.022	OR =0.52, p=0.522
LVEF (<50%)	OR =1.65, p=0.010	OR =0.35, p=0.611
PAP (<22mm)	OR 0.510, p=0.322	OR =0.41, p=0.427

DISCUSSION:

In our study 2 (4%), 6 (12%) and 42 (84%) patients had mild, moderate and severe OSA respectively. The higher prevalence of severe disease in the present study is probably due to hospital based referral bias model of the study as patients seek advice only in late stages.

Normal LVEF of ≥50% was present in majority (n=46) of the patients. Mild dysfunction with LVEF of 40-49% was present in 4 patients and all of them had severe OSA. In a study by Niromound et al, LVEF <60% was present in 45 out of 883 patients. They concluded that OSA in the absence of hypertension and obesity was not associated with decreased LVEF. Other studies have also confirmed that reduced LVEF was absent in mild to moderate form of the disease. [3],[4]

The cause of decreased LVEF is multifacto-rial and it may be due to hypertension, obesity or OSA. A binary logistic regression analysis of our study indicated that LVEF reduction was more related to hypertension than to OSA. DD was not common in patients with OSA. Only 6 patients had DD and all of them had severe OSA. Binary logistic regression analysis of DE parameters revealed that DD was related to hypertension than to OSA. Several pathophysiological mechanisms have been postulated for the development of DD in patients with OSA like Left Ventricular hypertrophy, activation of sympathetic nervous system and hypertension. [5]

Danica et al [5] in their study concluded that the prevalence of DD was higher in patients with OSA than in the general population. In the study done by Baguat et al, DD was present in 32 (22.8%) out of 150 patients with OSA and 81% of those with DD were hypertensive.[6] Echocar-diographic studies have shown both systolic and diastolic dysfunction with increasing AHI. [7]

Kanwar et al reported that the severity of DD was associated with increasing AHI. Diastolic dysfunction was present only in severe OSA with AHI

>30/hour. [8]

TR was absent in majority (36 out of 50) of our OSA patients. Patients with mild and moderate OSA did not have TR. Only 14 out of 42 patients with severe OSA had TR. This indicates that TR is not common in OSA. Because of small numbers of patients with mild and moderate OSA, we could not conclude whether progressively increasing OSA severity was associated with progressively increasing presence of TR. Moro et al reported TR in 4% of patients with OSA.[2]

OSA was not associated with PAH in most of our patients. Only six out of fifty patients had PAH. PAH was seen only in patients with severe OSA. All these patients had mild PAH. This indicates that OSA is not usually associated with PAH and if PAH occurs, it is of mild degree.

Studies done by Sanner et al and Bady et al also did not find a high prevalence of PAH in patients with OSA. They reported a DE determined mild rise in PAP (20-26 mm Hg) in 18/92 (20%) and 12/44 (27%) patients respectively. [11],[13] Sajkov et al found PAP >22 mm Hg in 11/27 (41%) patients. However, they did not find any correlation with the severity of OSA. [12] Fisher et al in their study compared PAP diagnosed by DE with right heart catheterization (RHC). They reported that the magnitude of pressure underestimation (33%) was greater than overestima-tion (13%) by DE. [10]

Patients with OSA have higher prevalence of hypertension. [3],[7] In our study, 31 (62%) OSA patients were hypertensive. Both OSA and hypertension are known to cause DE changes. DE changes in heart were not common in patients with OSA without hypertension. DE parameters such as DD, decreased LVEF were more related to hypertension, which is not uncommon in patients with OSA, than to OSA. Verbraecken et al also did not find DE changes in patients with OSA without hypertension[3].

In our study patients with OSA had mostly normal DE. It is possible that with a longer duration of untreated OSA, cardiac changes would occur. Most of our patients had symptoms of OSA ranging from a few months to a few years. Patients should, therefore, be followed up longitudinally to assess the effect of OSA on heart.

Most of our patients had severe OSA. This could be because of a hospital based referral bias of the study. A better comparison of cardiac parameters would have been achieved with adequate numbers of patients with mild and moderate OSA.

Only 6 patients in our study had PAH and all these patients had mild PAH. The Gold standard test for the diagnosis of PAH is RHC. Fisher et al in their study compared PAH measured by DE with that measured by RHC. They reported inaccuracy of DE in diagnosing PAH in 48% of their patients. This could be because of suboptimal visualization of Doppler signal across the tricuspid valve.

CONCLUSION:

Normal DE parameters were present in majority of the patients with OSA of all severity. Severe OSA, in some cases, was associated with abnormal DE. The DE changes that were present in these patients were more related to hypertension than to OSA per se.

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Evaluation of Liver Function Test and Renal Function Test in Pre-eclampsia: A Case Control Study

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Original Research Article

ABSTRACT

Pre-eclampsia is a multisystem disorder, which occurs only in pregnant women during the second and third trimesters of pregnancy and is associated with raised blood pressure and proteinuria. Liver function Test (LFT) abnormalities occur in 3% of the pregnancies and probably the lesion that causes elevated serum liver enzymes. With severe renal involvement, glomerular filtration may be impaired and the plasma creatinine concentration begins to rise. This study was conducted to compare the liver function tests and renal function tests in pre eclampsia with normal pregnancy. This study was carried out on 60 pregnant women after 20 weeks of gestation admitted in Obstetrics & Gynaecology units of Shri Shankaracharya Institute of Medical Sciences, Bhilai, Chhattisgarh. The subjects were divided into two groups. Group A comprised of 30 cases of pre-eclampsia having blood pressure ≥ 140/90mm Hg, proteinuria in 24 hours ≥ 300 mg and edema. Group B had 30 normal pregnant women after 20 weeks of gestation. The data including parity, period of gestation, blood pressure and presenting complaints of all subjects were recorded. The mean value of serum bilirubin in cases was 3.45 and in controls it was 0.50. The mean value of enzymes ALT in cases was 92.7 while in the controls it was 22.37. Mean serum AST in the cases was 85.43 and in the controls it was 21.96. Total protein in cases was 7.77 and controls it was 7.26. Albumin level in cases was 4.62 and controls were 4.17. The mean value of urea in cases was 43.76 and control it was 24.5. The mean value of Creatnine in cases was 1.51 and in control it was 0.80. The mean value of Uric Acid in cases was 5.41 and in control it was 4.45. Increased concentrations of serum bilirubin, total protein, albumin and liver enzymes ALT, AST,urea, creatinine and uric acid were found in pre-eclampsia cases.

KEY WORDS: hypertension, liver function test (LFT), pre-eclampsia, renal function tests (RFT)

INTRODUCTION:

Hypertension is the most common medical disorders in pregnancy[1]. Abnormal liver function test (LFT) occurs in 20% to 30% of pregnancies complicated by Pre-eclampsia and are associated with poor maternal and fetal outcomes[2,3]. Pre-eclampsia and eclampsia are pregnancy induced hypertensive disease[4].

Pregnancy Induce Hypertension (PIH) is raised blood pressure without proteinuria during the second half of pregnancy. Pre- eclampsia is a multisystem disorder, unique to pregnancy that is usually associated with raised blood pressure and proteinuria after 20 weeks of gestation. Eclampsia is one or more convulsions in association with syndrome of pre-eclampsia[5,6]. In pre-eclampsia the systolic BP is ≥ 140 mm Hg and diastolic BP ≥ 90mm Hg in a woman with previously normal blood pressure and with proteinuria ≥ 0.3gm in a 24 hour urine collection[5]. The main cause of pre-eclampsia is vasoconstriction and thickening of vascular media which decreases vascular capacity and increases peripheral resistance. The factors that appear to have role include placenta, maternal immune response, maternal vascular disease, genetic predisposition and maternal low calcium level. The cellular cause of pre-eclampsia lies within the placenta and resolution of pre-eclampsia starts with removal of placenta at delivery. In pre-eclampsia the ratio of prostacy-cline–thromboxane production rate is decreased favoring the vasoconstrictive thromboxane. During preeclamptic pregnancy, the placenta is under oxidative stress with increased production of lipid peroxides and decreased production of antioxidants.

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Maternal circulating oxidized lipids may be the cause of endothelial cell activation[7].

Liver function Test (LFT) abnormalities occur in 3% of the pregnancies and pre-eclampsia is the most frequent cause. In the last trimester liver disease associated with abnormal liver function tests, nausea and/or vomiting and abdominal pain is due to severe pre-eclampsia, HELLP syndrome or acute fatty liver of pregnancy with or without sub-capsular hepatic hematomas, amongst which there is an ovelap[8].

Liver dysfunction during pre-eclampsia has serious consequences. In pre- eclampsia accompanied by HELLP syndrome, an elevation in liver function test result is noted. Alanine aminotransferase (ALT) and Aspartate aminofransferase (AST) may also be elevated and hyper-bilirubinemia may occur, especially in the presence of haemolysis. Periportal hemorrhagic necrosis in the periphery of the liver lobule is probably the lesion that causes elevated serum liver enzyme levels. Haemorrhage under the liver capsule can be so severe that the capsule ruptures and causes life threatening intra peritoneal bleeding[8].

In normal pregnancy there is decreased blood pressure response to pressor substance but in pre-eclamptic there is marked response to vasopressin, epinephrine end angiotensin. This response of arterial system leads to generalized vasoconstriction and hypertension in preeclampsia. Generalized vasoconstriction is responsible for decreased Glomerular filtration Rate (GFR) and renal plasma flow. This causes alteration in urea, creatinine and uric acid levels[15].

The objective of this study was to compare liver function tests & renal function tests in pre-eclamptics and normal pregnant females.

MATERIAL AND METHODS:

This study was carried out on 60 pregnant women after 20 weeks of gestation admitted in Obstetrics & Gynaecology units of Shri Shankaracharya Institute of Medical Sciences, Bhilai, Chhattisgarh from July 2017 to July 2018 with convenience sampling. The subjects were divided into two groups. Group A comprised of 30 cases of pre-eclampsia having blood pressure ≥ 140/90mm Hg, proteinuria in 24 hours ≥ 300 mg and edema. Group B had 30 normal pregnant women after 20 weeks of gestation. The data including parity, period of gestation, blood pressure and presenting complaints of all subjects were recorded.

Serum bilirubin, total protein, albumin, ALT, AST, urea, creatinine & uric acid were measured by MERIL AUTO QUANT 200i analyzer.

Total and direct bilirubin was measured by Diazo method. Total protein by Biuret method (end point). Albumin by BCG dye method (end point). AST & ALT was measured by IFCC Kinetic method. Urea was measured by Urease-GLDH method. Creatinine by Jaffe's method. Uric acid by Uricase method.

Both the cases and controls were in the age group 15-45 years. Those with a major systemic disease which may elevate the patient's blood pressure or which may change the liver function tests eg. renal diseases, liver diseases, diabetes and cardiac disease were excluded. Patients using any drugs that affect liver function were not included.

Verbal and written consent was obtained from each subject. Complete obstetrical and family history was recorded on proforma designed for the study.

Four variables were measured for all cases and controls ie. Serum bilirubin level, total protein, albumin and plasma levels of liver enzymes ALT, AST were measured using auto quant Meril 200 analyzer. Mean and standard deviation were calculated. The data were calculated.

The data was analyzed using SPSS-16. The mean values were compared between cases and controls using t-test at 5% level of significance.

RESULTS :

The mean value of serum bilirubin in cases was 3.45 and in controls it was 0.50. The mean value of enzymes ALT in cases was 92.7 while in the controls it was 22.37. Mean serum AST in the cases was 85.43 and in the controls it was 21.96. Total protein in cases was 7.77 and controls it was 7.26. Albumin level in cases was 4.62 and controls were 4.17.

The mean value of urea in cases was 43.76 and control it was 24.5. The mean value of creatnine in cases was 1.51 and in control it was 0.80. The mean value of Uric Acid in cases was 5.41 and in control it was 4.45.

The LFT & RFT variables were noted on cases as well as control. All the variables are continuous in nature. It has been observed that cases and controls were matched for age and there was no significant difference found in age of cases and control. Total bilirubin levels were found to be higher in cases compared to control. The difference of total bilirubin turned out to be highly significant statistically. Direct bilirubin readings show that the

Table 1: Liver Function Test (lft)

	Control Mean(SD)	Cases Mean(SD)	t – statistic	p – value
Age	29.23(6.08)	27.53(5.15)	1.16	0.24
Total bilirubin	0.503(0.19)	3.45(0.78)	-20.02	0.00001*
Direct bilirubin	0.282(0.05)	1.58(0.34)	-20.66	0.00001*
Indirect bilirubin	0.302(0.09)	4.02(1.68)	-12.06	0.00001*
SGPT	22.37(6.97)	92.7(19.91)	-18.25	0.00001*
SGOT	21.96(5.43)	85.43(13.65)	-23.64	0.00001*
TP	7.26(0.65)	7.77(0.91)	-2.44	0.01785*
Albumin	4.17(0.4)	4.62(0.95)	-2.37	0.02251*
Globulin	2.77(0.46)	3.12(0.73)	-2.22	0.0308*

*Statistically significant

Table 2: Renal Function Tests (RFT)

	Control Mean(SD)	Cases Mean(SD)	t – statistic	p – value
Urea	24.5(6.01)	43.76(6.03)	-12.38	0.00001*
Creatinine	0.80(0.09)	1.51(0.18)	-18.62	0.00001*
Uric Acid	4.45(1.09)	5.41(0.84)	-3.8	0.00035*

*Statistically significant

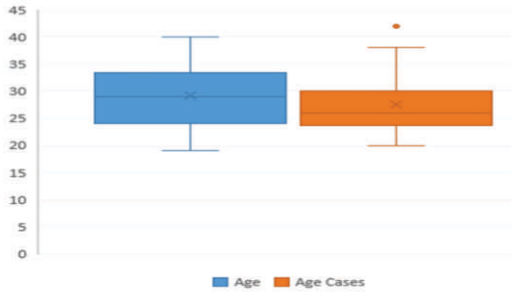

Figure 1: Box and Whisker plot for age distribution.

levels were elevated in cases and the difference between levels of direct bilirubin among cases and controls turned out to be highly significant. Indirect bilirubin was found to be significant and the readings were observed to be higher in cases. SGPT and SGOT were found to be very high in cases and the difference was statistically highly significant. TP levels were slightly elevated in cases and the difference was statistically significant. In case of albumin and globulin the observations were recorded higher in cases compared to control. Albumin and globulin were found to be highly significant. Urea and Creatinine were highly signigicant in cases when compared to controls.

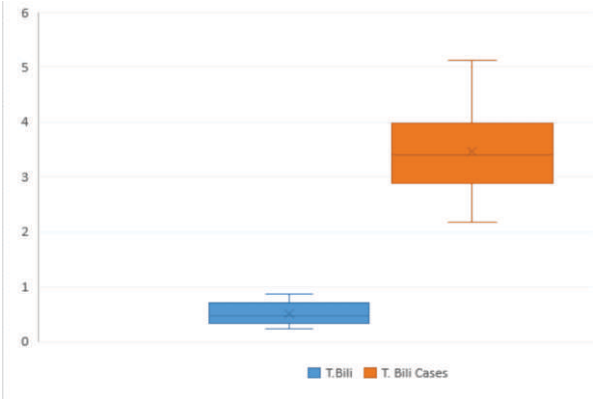

Figure 2: Box & whisker plot for total bilirubin among control and cases

Figure 3: Box & whisker plot for direct bilirubin among control and cases.

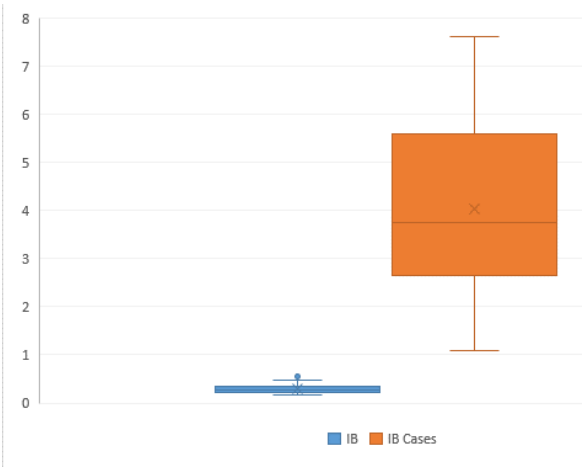

Figure 4: Box & whisker plot for indirect bilirubin among control and cases.

DISCUSSION:

Hypertensive disorders complicating pregnancies are common now a days and consists of both eclampsia and pre-eclampsia. Pre-eclampsia is a condition that develops in previously normotensive pregnant women after 20 weeks of gestation and is

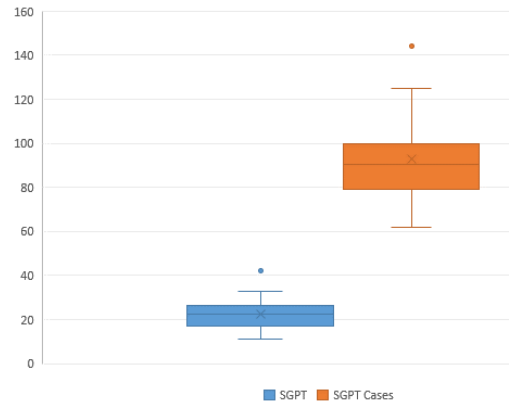

Figure 5: Box & whisker plot for SGPT among control and cases

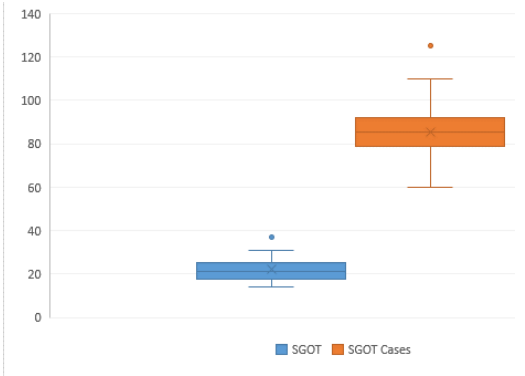

Figure 6: Box & whisker plot for SGOT among control and cases.

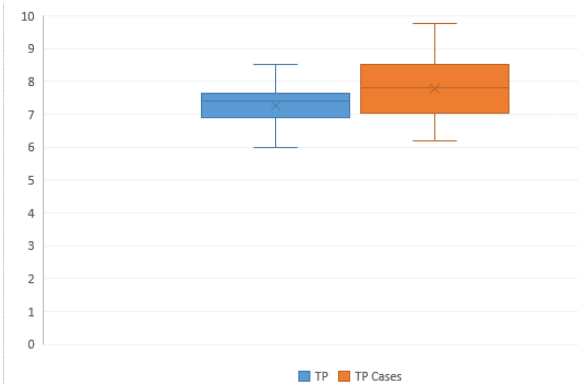

Figure 7: Box & whisker plot for TP among control and cases.

characterized by onset of hypertension and proteinuria. Pre-eclampsia can affect every maternal organ, predominantly the vascular, renal, hepatic, cerebral and coagulation system[5,9,10]. Concentration of serum bilirubin in the present study was significantly higher ($p < 0.001$) in patients of pre-eclampsia before delivery than the control group of same age and parity with normal blood pressure. Malvino et al. showed that in HELLP syndrome serum

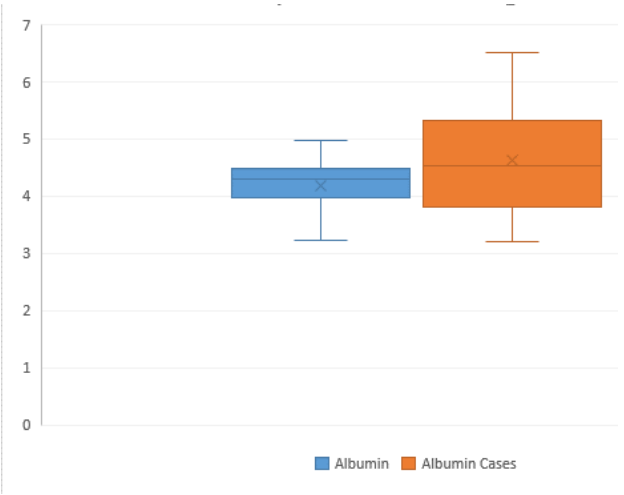

Figure 8: Box & whisker plot for albumin among control and cases.

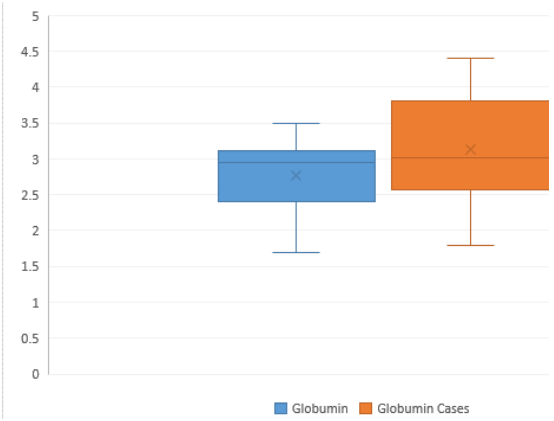

Figure 9: Box & whisker plot for Globumin among control and cases.

bilirubin concentration was elevated from its normal value to about > 1.2 mg/dl[11]. Similarly Jaleel et al. noted that there was a highly significant rise in serum bilirubin, lactate dehydrogenase and aspartate aminofransferase level in pre-eclamptic women compared to normotensive pregnant women.[12] Serum ALT of pre-eclamptic women in the present study was significantly ($p<0.001$) elevated from their normotensive pregnant counterparts. Malvino et al. observed that in pre-eclampsia the serum transaminase level was raise to >10 U/L and that of ALT to 271±297 U/L[11].

In the present study the mean serum AST level in pre-eclamptic cases was found significantly higher ($p < 0.001$) than the normotensive control group. Serum AST level in pre-eclampsia was also found more than 70 U/L by Malvino et al, which rose upto 209±178 U/L in eclampsia[11].

Rath et al also noticed elevated level of ALT and AST in severe pre-eclampsia[13].

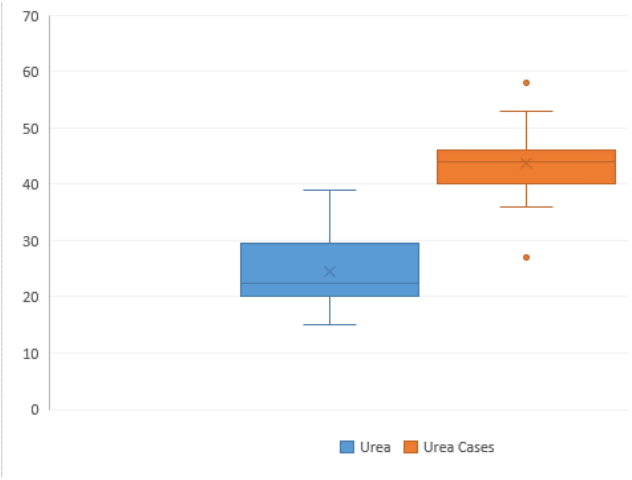

Figure 10: Box & whisker plot for Urea among control and cases.

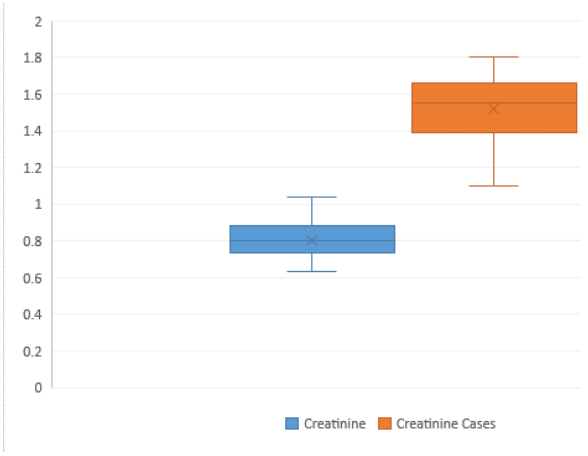

Figure 11: Box & whisker plot for Creatinine among control and cases.

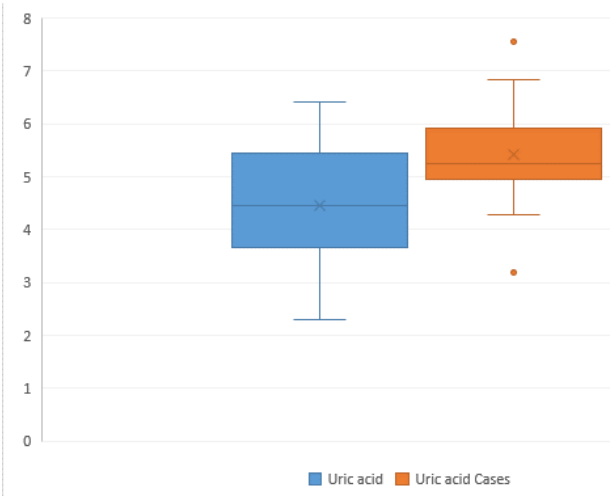

Figure 12: Box & whisker plot for Uric Acid among control and cases.

Mechanism of raised liver enzymes hypervascularisation and vaso constriction of liver leading to cell injury, alteration of membrane permeability & damage to hepatocytes. Liver dysfunction during pre-eclampsia has serious consequences. In pre- eclampsia accompanied by HELLP syndrome, an elevation in liver function test result is noted. Alanine aminotransferase (ALT) and Aspartate aminofransferase (AST) may also be elevated and hyper-bilirubinemia may occur, especially in the presence of haemolysis. Periportal hemorrhagic necrosis in the periphery of the liver lobule is probably the lesion that causes elevated serum liver enzyme levels. Haemorrhage under the liver capsule can be so severe that the capsule ruptures and causes life threatening intra peritoneal bleeding[8].

In normal pregnancy there is decreased blood pressure response to pressor substance but in pre eclamptic there is marked response to vasopressin, epinephrine end angiotensin. This response of arterial system leads to generalized vasoconstriction and hypertension in preeclampsia. Generalized vasoconstriction is responsible for decreased Glomerular filtration Rate (GFR) and renal plasma flow. This causes alteration in urea, creatinine and uric acid levels.

In our study the mean gestation age of pre-eclamptic patients was 35.4 ± 4.18 weeks. This is in agreement with Kim et al. who noted that among the abnormal liver function tests in pregnancy 39.4% occurred between 30 and 40 gestational weeks while 29% occurred between 10 and 20 weeks and common causes were hyperemesis gravidarum followed by pre-eclampsia; viral hepatitis and HELLP syndrome[14].

Renal Function Test (RFT) like urea and creatnine are significantly elevated. Serum uric acid is not increased significantly. Serum urea, creatnine is significantly elevated ($p > 0.001$) in Jlmaah et al.[16] Some investigators found that the activity of Monoamine Oxidance (MAO) is lower and serotonin is higher. Reduced GFR with reduced plasma renal blood flow causes increased serum creatinine and blood urea Hussein el al[17].

In the present study there is significant increase in urea and creatinine which has been interpreted to act as important co-factor in the pathogenesis of pre-eclampsia. Present study also suggests that serum liver enzymes, bilirubin, total protein, urea, creatnine to be of immense value in undertaking the pathogenesis and also appears to be important factor deciding pregnancy.

CONCLUSION:

In the present study, serum levels of total bilirubin , SGOT, SGPT, total protein were significantly raised in the cases compared to controls. During pre eclamptic pregnancy, the placenta is under oxidative stress with increased production of lipid peroxides along with anti oxidants. Maternal circulating oxidized lipids may be the cause of endothelial cell destruction leading to liver cell injury. These results in abnormal liver function tests. More studies are required to substantiate our hypothesis.

Liver and Renal involvement in Pre-eclampsia is very common in co relation to oxidative stress, deranged plasma renal blood flow leading to decreased GFR and increased renal parameters to combat with marker of oxidative stress.

Present study will be helpful to determine the status of the working of kidney and liver to plan the definitive and proper treatment of pre eclampsia along with management of liver and kidney parameters.

LIMITATION OF THE STUDY:

The limitation of our study is that the study subjects are less & should be done on a larger population.

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Transition of Vivax Malaria from Benign to Malignant Clinical Profile: A Cause of Concern

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Original Research Article

ABSTRACT

This present research aimed to study hepatic dysfunction, renal dysfunction and prevalance of thrombocytopenia in children with malaria in tertiary care centre of Bundelkhand region. Children in the age groups of 1 to 15 yrs, whose peripheral smear were positive for malarial parasite were included in the study group from amongst those admitted in pediatric ward of tertiary care hospital in Jhansi. A detailed history and clinical examination was done, followed by investigations like hemogram, platelet count general blood picture, reticulocyte count, serum bilirubin, SGOT, SGPT, serum alkaline phosphatase, blood urea and serum creatinine. Raised level of SGPT, SGOT and total serum bilirubin were seen in 31%, 30% 53% respectively. In our study 11% of cases had raised level of blood urea or serum creatinine or both. Greatest affected group was 10-15 yrs in which 6 cases had increased urea and creatnine level. There were 88 cases (88%) in which platelet count was < 1.5 lac/Cu mm, among them P.falciparum cases were 47(47%) and P.vivax cases were 41(41%). Liver functions are commonly affected in malaria and liver dysfunction ranges from mild elevation of liver enzymes to the range of acute hepatitis . There was no significant difference in incidence of thrombocytopenia between vivax and falciparum cases. Mild renal impairement was present in a number of cases and it was noted that dehydration also plays role in the genesis of renal impairement in children with malaria.

KEY WORDS: falciparum, liver dysfunction, renal dysfunction, thrombocytopenia

INTRODUCTION:

Malaria due to P.vivax had been considered to have benign course. It is known for multiple relapses and falciparum infection was associated with complicated malaria. However in the past few years there is a changing trend in the clinical manifestations of vivax malaria from severe or complicated disease to sometimes even causing death. Hepatic dysfunction is usual in severe malaria. Jaundice is common, other like reduction in clotting factor synthesis, metabolic clearance of drugs, biliary excretion, and failure of gluconeogenesis contributes to lactic acidosis and hypoglycaemia.

The activity of a broad range of cytochrome p450 mixed function oxidase is reduced including cyp3a4 which is responsible for quinine 3 hydroxylation which is the principle route of quinine metabolic clearance, that is reduced in proportion of disease severity[1]. Jaundice in malaria has haemolytic, hepatic, and cholestasis components. Choestasis jaundice may persist well into the recovery period. Liver damage may result from an alteration in vascular flow through the organ as the parasitized RBCs adhere to endothelial cells, blocking sinusoids and obstructing intrahepatic blood flow. Intravascular hemolysis of parasitized and nonparasitized RBCs has been considered as an important factor in the causation of mild to moderate jaundice, in which bilirubin is predominantly unconjugated. However, hemolysis is never the sole cause of severe jaundice nor the conjugated hyperbilirubinemia . Increase in the serum levels of aspartate amino transferrase (AST) and alanine amino transferase (ALT) are seen in many patients. In severe malaria renal microvasculature obstruction occurs due to sequestration in the kidneys. Impaired perfusion may be compounded by reduced erythrocyte deformability and the release of significant amount of haemoglobin and cellular debris during hemolysis. Massive hemolysis accounts for black water fever complicating severe malaria.

Thrombocytopenia is common among people indigenous to the tropics, and non- immune subjects infected by Plasmodium falciparum or P. vivax .

Malaria-related thrombocytopenia may result from either a decrease in palatelet production or an

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increased platelet turnover due to different mechanisms of destruction.

It has been stated in different literature about various immunological and nonimmunological destruction of platelet leading to thrombocytopenia[2].

MATERIAL AND METHODS:

The present study was conducted in the Department of paediatrics with active collaboration of the department of pathology. Children between age group of 1 to 15 years, who were admitted to paediatrics ward of this hospital from June 2016 to july 2017 were studied.

This study included patients in whom a diagnosis of malaria were confirmed by the presence of malaria parasite in the blood either a smear positive for plasmodium species and/or malarial antigen positive by RDT (rapid diagnostic test). Suspected cases of smear negative malaria and RDT negative were excluded from the study. Study plan was approved by hospital research committe.

A detail history was taken and clinical examination was done to rule out any past history of renal, cardiovascular, hepatic, endocrine, or metabolic disorder. The history of fever, its duration and severity, vomiting, loose motion, headache, convulsion, abdominal pain, hemoglobinuria, jaundice, bleeding manifestation, any h/o malaria were recorded.

Patient were examined for dehydration, pallor, hypotension, CNS manifestation, jaundice, edema, respiratory distress, evidence of bleeding. Liver size, spleen size, and urine output were noted. The patients were then subjected to thorough systemic examination.

Diagnostic methods used were conventional thick and thin peripheral smear stained with Leishman stain, examined under oil immersion. Rapid diagnostic test were based on detection of specific plasmodium antigen. Other lab investigation were undertaken like hemogram, platelet counts, general blood picture, recticulocyte count, serum bilirubin (total and direct), SGOT, SGPT, serum alkaline phosphatase, blood urea, serum creatinine, urine for routine examination.

About 8 ml of blood was collected by venipuncture from every patient after correction of dehydration if present taking due aseptic precaution. Statistical analysis was done.

RESULTS:

In this study maximum number of patient affected were in the age group of 5-10 yrs and the least affected group was 1-5 yrs, which was 38% and 25 % respectively. 37% of affected children were between the age of 10-15yrs.

Total number of P.falciparum affected patient were 58 (58%), and P.vivax positive cases were 42(42%). P.falciparum cases were maximum 27(27%) in the age group of 10-15 years. P.vivax affected cases were 17%, 15%, 10% in the age groups of (1-5), (5-10), (10-15) yrs respectively.

In this study, it was found that malaria can present with a wide range of symptoms. The number of cases presented with fever were 99 (99%) and 1% case was without fever. P.falciparum cases with fever were 57 (57%), whereas P. vivax cases with fever were 42 (42%). Total 16% of cases presented with abdominal pain, of which 10% were P. falciparum and 6% were P.vivax positive cases. Among all cases 5% had diarrhea and 24 (24%) had convulsions, in which 17 (17%) were P. falciparum positive and 7 (7%) cases were P. vivax positive. Jaundice was seen in 18 (18%) cases at the time of admission in our hospital, in which 8 (8%) were P. falciparum positive and 10 (10%) were P.vivax positive . Bleeding manifestation was noted in 19 (19%) cases, in which 13(13%) were P.falciparum and 6% were P.vivax positive.

The number of cases those presented with decreased urine output were 11% and 5% of total cases presented with cough. Edema was present in 5% of total cases in which 3% were P. vivax and 2% cases were P.falciparum positive. There were 43 (43%) cases who had pallor among which 30 (30%) were P.falciparum and 13 (13%) were P.vivax positive. Two (2%) cases had signs suggestive of Congestive heart failure. Single Case (1%) was noted with black water fever.

There were 92% (92) cases who had splenomegaly/ hepatosplenomegaly/ hepatomegaly. Isolated hepatomegaly was present in 9% of total cases, whereas isolated splenomegaly was present in 21% of total patient. The number of patient those had hepatosplenomegaly were 62(62%) .

The number of cases whose haemoglobin level were between 6-9.9 gm% were 34 or 34% of total, 20 cases of them were P. faciparum and 14 cases were P.vivax positive. 24% of patients had haemoglobin level between 10-12gm% and 4% cases have Hb level of > 12gm% . It was observed that 27% of cases who had Hb% <6gm were P. falciparum cases and 11% cases were vivax positive .

In this study 53%(53) of cases had increased level of total serum bilirubin (>1mg%). The number of P. falciparum and P.vivax infected person who had increased level of total serum bilirubin were 29(29%)

Table 1: Number of cases and parasite distribution in each age group.

Age group	1-5 yrs	5-10 yrs	10-15 yrs	Total
No. of cases	25%	38%	37%	100%
P.vivax	17%	15%	10%	42%
P. falciparum	8%	23%	27%	58%

Table 2: Presentation of malaria

Symptoms/signs	P.falciparum	P. vivax	Total
Fever	57	42	99
Without fever	1	0	1
Abdominal pain	10	6	16
Diarrhea	2	3	5
Convulsion	17	7	24
Jaundice	8	10	18
Bleeding	13	6	19
Oliguria	5	6	11
ARI	2	3	5
Edema	2	3	5
Moderate to severe pallor	30	13	43
CHF	1	1	2
Black water fever	1	0	1

Table 3: Correlation between hepatosplenomegaly, Hb%, Serum bilirubin, SGOT, SGPT , Thrombocytopenia, renal functions and type of malaria.

Parasite	P.falciparum	P.vivax	Total
Hepatosplenomegaly	32	30	62
Hepatomegaly	4	5	9
Splenomegaly	15	6	21
Hb%			
<6gm%	27	11	38
6-9.9gm%	20	14	34
10-12gm%	16	8	24
>12gm%	3	1	4
S.bilirubin (>1mg%)	29	24	53
SGOT (>80U/L)	19	11	30
SGPT (>80U/L)	20	11	31
Platelet count			
<50,000	22	18	40
50,000 -1lac	13	11	24
1-1.5lac	12	12	24
Renal functions			
S.urea (>40mg/dl) S.creatinine(>1mg/dl)	5	6	11

Table 4: Correlation between raised blood urea, raised serum creatinine and type of malaria.

Age group	1-5yrs	5-10yrs	10-15yrs	Total
P.vivax	0	1	4	5
P.falciparum	1	3	2	6
Total	1	4	6	11

and 24%(24) respectively. Most affected age group was 5-10 yrs in which 22% cases had increased level of serum bilirubin and least affected group was 1-5 yrs in which 13 % of patients had increased level of total serum bilirubin.

It was observed that the total number of cases in whom raised level of SGPT(>80IU/L), SGOT (>80IU/L) and total serum bilirubin seen were 31 %, 30% ,53% respectively. Highest number of cases who had increased level of liver enzymes and serum bilirubin were seen in P. falciparum cases, which was 20%, 19%, 29% for SGPT,SGOT and serum bilirubin respectively. There was no stastically significant difference between these elevated SGPT, SGOTand serum bilirubin in P.falciparum and P.vivax cases.

In our study, 88 cases (88%) had platelet count < 1.5 lac/mm3 . P.falciparum cases who had platelets counts < 1.5 was 47 (47%) and rest were P. vivax positive cases which were 41 (41%) . Cases in which platelets counts were less than 50000 were 40 (40%) of total cases, where 22 (22%) cases were P. falciparum positive and 18(18%) were P.vivax positive. There were 24 (24%) cases in whom platelets counts was between 0.5-1 lac and 24 (24%)cases had platelet counts between 1-1.5lac/mm3 .

In Our study 11 (11%) of cases had raised level of blood urea (40mg/dl) or serum creatinine (1mg/dl) or both. Greatest affected group was 10-15 yrs in which 6 cases had increased urea and creatinine level, next was age group of 5-10 years and least affected was 1-5 years age group.

DISCUSSION:

This study was conducted in tertiary care hospital of Jhansi which caters to a vast area of Bundelkhand region which is endemic for malaria. This study was a modest attempt to study the changes in liver and renal functions along with occurence of thrombocytopenia in children affected with malarial parasite.

In this study 9% of cases presented with hepatomegaly, 21% with isolated splenomegaly. Overall hepatomegaly /splenomegaly/ hepatosplenomegaly was present in 92% of the cases which correlated with study of Nityanand et al[3].

It was observed that 88% of cases had platelet count below 1.5 lac/mm3, 64% cases below 1 lac/Cumm, 40% cases below 50000/mm3.

Platelets were diminished in association with intravascular coagulation. It took 5-7 days after start of treatment before it retrun to normal. Thrombocytopenia is a common finding in faciparum malaria and may be due to sequestration of platelets in capillaries of internal organs. It was seen many times in patients with vivax malaria without severe forms also had thrombo-cytopenia.

Among the cases of malaria, 53% had elevated level of serum bilirubin of which 29% were of p. falciparum and 24% of P.vivax. This was in concordance with the study by Patwari et al[4] where serum bilirubin was raised in 26% of cases infected with P.vivax. Serum bilirubin was also shown to be increased in many other studies by Mishra et al[5] and Goyal et al[10].

In our study SGPT was raised in 31% cases and SGOT was raised in 30% of cases which is higher than those reported by Bag et al[6] may be, it was astudy of small series of 16 cases of falciparum malaria .

Renal impairement was depicted in our study as raised levels of blood urea and serum creatinine in 11% of the cases . Ahmad et al[7] reported impaired renal function in 48% of cases of total malaria out of which 66% accounted for falcipuram malaria 30% for vivax malaria. Habte et al[8] reported acute renal failure in 33% of patients of severe malaria whereas Weber and boker et al[9] reported acute renal failure in 29.03% of falciparum malaria cases in one series. In one of the study impaired renal functions were reported in 12% of P.falciparum and 8.4% of P.vivax cases that supported our study[13].

The incidence of malaria varies from study to study and this variation remained unexplained and probably depends on individual susceptibility. The disturbance in renal microcirculation is responsible for acute renal failure and immunological reaction to parasites accounts for glomerular lesion as stated in study by Sitprija et al[12]. In our study none of the patients required dialysis due to early recognition of impaired renal function and aggressive management thus improvement in renal microcirculation.

It was also seen that there was statistically no significant difference between hepatic dysfunctions, renal impairment and incidence of thrombocytopenia between P.falciparum and P.vivax cases in our study. The study by Goyal et al[10] supports it as they also concluded no such statistical difference. Kumar et al[11] also concluded impairments in liver and renal functions equally high in both the types of malaria in different age groups with no significant difference between these groups.

CONCLUSION:

To conclude it was found that there was statistically no significant difference between the hepatic dysfunctions, Renal dysfunctions and incidence of thrombocytopenia in both the types of malaria (P.falciparum and P.vivax). The hepatic dysfunction ranges from mild elevation to the range of acute hepatitis. Mild renal impairment was present in a number of cases and it was seen that dehydration also plays a role in the genesis of renal impairement in children with malaria.

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Elevated Vitamin B_{12}: An Indicator of Severity among Cirrhotics

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Original Research Article

ABSTRACT

Concentrations of cyanocobalamin (vitaminB12) in serum have been found to be elevated in acute and chronic liver disease associated with hepatocellular damage.In severe liver disease, liver tissue B12 binding is disrupted and causes B12 to leak out of the liver into the circulation. The cross sectional study was conducted on 50 cirrhotic patients presenting to OPD or admitted in the Department of Medicine, People's Hospital, Bhopal, from November 2017 to April 2018 with objective to estimate serum vitamin B12 levels in patients with chronic liver disease and to find out the relationship between severity of cirrhosis (Child Pugh) with levels of serum Vitamin B12. The inclusion criteria was age>18 years and known and established cases of cirrhosis by ultrasound abdomen. Patients with the history of chronic alcoholic liver disease and patients taking vitamin B12 supplements. Details regarding socio-demographic variables and history was obtained followed by physical, biochemical and histological examination of liver. Majority of the patients belonged to the age group 41-60 years (48%) and 74.0% were males. Mean Vitamin B 12 level in present study was estimated to be 518.2±245.4 pg/ml. The association of mean Vitamin B12 in relation to Child Pugh Class was found to be statistically significant ($p<0.05$). Vitamin B 12 levels in present study were found to be elevated amongst the patients of cirrhosis. Also the association of mean Vitamin B12 in relation to Child Pugh Class was found to be statistically significant ($p<0.05$).

KEY WORDS: vitamin B_{12}, child pugh score, cirrhosis

INTRODUCTION:

Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver injury, that leads to portal hypertension and end stage liver disease. This is accompanied by distortion of the hepatic vasculature. It leads to shunting of the portal and arterial blood supply directly into the hepatic outflow (central veins), compromising exchange between hepatic sinusoids and the adjacent liver parenchyma.[1]

Vitamin B12 is stored primarily in the liver. It acts as a cofactor for two enzymatic reactions, namely, methionine synthesis from homocysteine and succinyl-CoA synthesis from methylmalonyl-CoA, in mammalian systems.[2] Concentrations of cyanocobalamin (vitaminB12) in serum have been found to be elevated in acute and chronic liver disease associated with hepatocellular damage.[3] In severe liver disease, liver tissue B12 binding is disrupted and causes B12 to leak out of the liver into the circulation. Eventually liver disease could produce enough severe tissue B12 deficits to cause metabolic dysfunction despite elevated plasma total B12.[4]Moreover, increased blood vitamin B12 concentration has recently been identified as a prognostic indicator for patients with hepatocellular carcinoma.

In acute viral hepatitis the rise in serum cyanocobalamin seemed to pronounced in the first two weeks of the disease, when bilirubinemia is marked and the liver-function tests indicative of hepatocellular damage are positive.[5] In cirrhosis of the liver elevated cyanocobalamin values were found when clinical and laboratory signs of active hepatitis were present. However normal levels are observed in extrahepatic obstructive jaundice.[3]Depletion of cyanocobalamin stores of the liver has been demonstrated in cirrhosis. This depletion may well be responsible for the development of macrocytic anemia in some cases of cirrhosis.[3]

Since the prevalence of cirrhosis in our country is high, the present study was undertaken to determine the levels of Vitamin B12 in patients with cirrhosis and find out if any correlations exists between the severity of cirrhosis and Vitamin B12.

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MATERIAL AND METHODS:

The present study was conducted as a cross sectional study on cirrhotic patients presenting to OPD or getting admitted in the Department of General Medicine, People's College of Medical Sciences, Hospital and Research Centre, Bhanpur Bhopal,for a period of 6 months from November 2017 to April 2018.

A total of 50 patients were selected using convenient sampling. The study population consists of known and established patients of Liver cirrhosis. The inclusion criteria was age>18 years and known and established cases of cirrhosis by ultrasound abdomen. Patients with the history ofchronic alcoholic liver disease, chronic kidney disease, diabetic patients, patient not willing for study and patients taking vitamin B12 supplements.

The study was approved by Institutional Ethics Committee. After obtaining the informed consent, socio-demographic details were obtained from the patient and entered in preformed pretested questionnaire. A detailed history was elicited, with particular attention to alcohol intake or intake of supplements, to assess the various risk factors for liver cirrhosis. Each patient was examined physically to assess the general condition and the vital data was recorded. Per abdominal examination was done according to the standard protocol and the findings were documented. The physical stigmata of cirrhosis and ultrasonographic findings (like nodular liver surface, coarse echotexture of liver parenchyma, splenomegaly, portal and splenic vein diameters, ascites etc.) was used for including cirrhotic patients in study and child pugh score was used to assess severity of cirrhosis.Based on the severity of the signs and symptoms and the USG reports, the treatment modality was decided. Biochemical analysis including Vitamin B12 level estimation and histopathology was also done.

Data was compiled using MS excel and analysed using Epi Info 7.2 software. Appropriate statistical tests were applied.

RESULTS:

A total of 50 patients were enrolled in present study. Majority of the patients belonged to the age group 41-60 years (48%) followed by 21-40 years (34%). There were 13 (26.0%) females, while 37 (74.0%) were males, showing a male preponderance in the study.

Majority of the patients were having coarse and altered liver echotexture (50%) followed by altered echotexture (38%). Majority of the patients were in the Child Pugh Class B (56%), followed by Class C (32%) whereas only 16% patients belonged to Child Pugh class A.

Table 1: Distribution of patients according to socio-demographic variables.

Socio-demographic variables		Frequency	Percentage
Age Group (years)	15-20	2	4.0
	21-40	17	34.0
	41-60	24	48.0
	61-80	6	12.0
	>80	1	2.0
Gender	Female	13	26.0
	Male	37	74.0

Table 2: Distribution of patients according to liver echotexture.

Liver Echotexture	Frequency (n=50)	Percentage
Altered	19	38.0
Altered with fatty infiltration	1	2.0
Coarse	1	2.0
Coarse and altered	25	50.0
Mild hepatomegaly	1	2.0
Raised echogenicity	1	2.0
Slight altered	1	2.0
Subtle altered	1	2.0

Table 3: Distribution of patients according to Child Pugh Class.

Child Pugh Class	Frequency	Percentage
Class A	8	16.0
Class B	26	56.0
Class C	16	32.0

Table 4: Comparison of mean Vitamin B12 in relation to Child Pugh Class.

Child Pugh Class	Frequency	Vitamin B 12 Mean	SD	f- value	p-value
Class A	8	320.85	177.07		
Class B	26	462.63	193.98	11.385	0.001*
Class C	16	707.18	234.96		

One-way ANOVA applied. P value = 0.001, Significant

Mean Vitamin B 12 level in present study was estimated to be 518.2±245.4 pg/ml.

In Class A, the mean vitamin B12 level was 320.85 ± 177.07 ng/ml, in Class B it was 462.63 ± 193.98 ng/ml and in Class C it was 707.18 ± 234.96 ng/ml. The mean Vitamin B12 was highest in the Child Pugh Class C and lowest in the Child Pugh Class A. The comparison of mean Vitamin B12 in relation to Child Pugh Class was found to be statistically significant ($p<0.05$). To find out the pairwise comparison, post hoc Tukey test was applied.

The pairwise comparisons were done between the pairs – Class A – Class B; Class A – Class C and

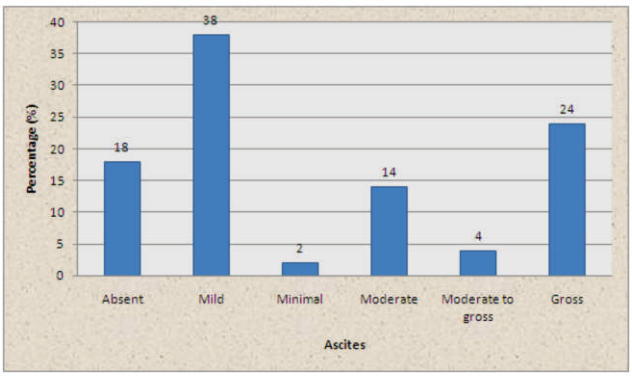

Figure 1: Distribution According to Ascites.

Post-hoc Tukey

Pair	p value	Interpretation
Class A – Class B	0.214	NS
Class A – Class C	0.000 *	S
Class B – Class C	0.001 *	S

* Significant

Class B – Class C. There was no statistically significant difference seen in the pair Class A – Class B ($p>0.05$), showing a comparable vitamin B12 level between the Class A and Class B.

The mean Vitamin B12 level was higher in the Child Pugh Class C in comparison to the Child Pugh Class A ($p<0.05$). The mean Vitamin B12 level was higher in the Child Pugh Class C in comparison to the Child Pugh Class B ($p<0.05$).

DISCUSSION:

Mean age of patients in present study was 46.1± 15.6 years and majority of patients (48%) belonged to 41-60 years of age followed by 21-40 years of age (34%). Majority of patients were male (74%) and only 26% were females. The mean age of patients with liver cirrhosis in a study by Port GZ et al (2014) was 60.7±10.8 years and majority of patients were males (56.3%).[6]

In present study, majority of the patients were having coarse and altered liver echotexture (50%) followed by altered echotexture (38%). Mild ascites was present in 38% patients and gross ascites in 24% patients. Cirrhotic ascitic fluid accumulation represents a very common manifestation of decompensated cirrhosis and results from a number of factors broadly defined in terms of hormonal and cytokine dysregulation and related volume overloading the setting of portal hypertension.[7]

Majority of patients in present study were in Child Pugh Class B (56%) followed by Child Pugh Class C (32%). In a study by Haq MI et al (2016), 17 (17%) patients were in Child Pugh class A and 35 (35%) were in Child Pugh class B and 48 (48%) were in Child Pugh class C.[8]

Amongst patients with Child Pugh Class A in present study, the mean vitamin B12 level was 320.85 ± 177.07 ng/ml, where as in patients with class B and Class C it was 462.63 ± 193.98 ng/ml and 707.18 ± 234.96 ng/ml respectively. The mean Vitamin B12 was highest in the Child Pugh Class C and lowest in the Child Pugh Class A. The comparison of mean Vitamin B12 in relation to Child Pugh Class was found to be statistically significant ($p<0.05$) in present study. These findings were similar to study by Sugihara T et al (2017) in which Serum vitamin B12 levels were significantly higher in patients with cirrhosis than chronic hepatitis [647 (160–2956) vs. 461 (189–2956) pg/mL ($p = 0.029$)]. In patients with Child-Pugh C, Child-Pugh A and Child-Pugh B, the mean B12 level - was 1308 ± 599 pg/mL, 784 ± 559 pg/mL, and 660 ± 464 pg/mL respectively. They also found statistically significant association between Vitamin B12 and Child Pugh Class ($p = 0.036$).[2] Bosy-WestphalA et al (2003) in their study observed that mean plasma folate was normal in patients with liver disease, but vitamin B-12 was elevated in cirrhosis.[9] In patients with Child-Pugh A, Child-Pugh B and Child-Pugh C class, the mean B12 level was 534 ± 384 pg/mL, 724 ± 506 pg/mL, and 1538 ± 918 pg/mL respectively.[9]

In present study, the association between Vitamin B 12 level was also observed between Child Pugh classes using post hoc Tukey test. No statistically significant difference was observed between patients of Child Pugh Class A – Class B ($p>0.05$) whereas the mean Vitamin B12 level was higher in the Child Pugh Class C as compared to the Child Pugh Class A ($p<0.05$). Also the mean Vitamin B12 level was higher in the Child Pugh Class C in comparison to the Child Pugh Class B ($p<0.05$). It was observed that, plasma concentrations of vitamin B-12 was elevated and increased with the severity of liver disease. A cellular leakage of vitamin B-12 with a subsequent intracellular vitamin B-12 deficiency has been proposed for liver cirrhosis.[10]

CONCLUSION:

Vitamin B 12 levels in present study were found to be elevated amongst the patients of cirrhosis. Also the association of mean Vitamin B12 in relation to Child Pugh Class was found to be statistically significant ($p<0.05$). Since elevated Vitamin B 12 level in patients of cirrhosis was associated with Child Pugh Score, it must be assessed in each patient of acute

or chronic liver disease.

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Health Facility Based Efficacy Assessment of In-use Disinfectants

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Original Research Article

ABSTRACT

Disinfection in hospital practice is mainly achieved either by surface disinfection or immersing the contaminated objects in the disinfectant solution. This study was undertaken for assessing the efficacy of commonly used disinfectants in a tertiary care teaching hospital against Multi Drug Resistant *Pseudomonas aeruginosa* isolate, and to evaluate the different methods of testing of disinfectants like Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test. In this study, samples of disinfectants and those in use, namely, Lysol, Sodium Hypochlorite, Glutaraldehyde and Bacillocid were collected from different sites in the hospital. The standard tests to check efficacy included Rideal-Walker Phenol Coefficient Test, Chick –Martin Test, Kelsey -Sykes Test, Surface Disinfection Test, In-Use Test. The tests were carried out by standard procedures. It was evident in the present study that Bacillocid had good efficacy in its working concentration.

KEY WORDS: bacillocid, disinfectants, efficacy, *pseudomonas aeruginosa*

INTRODUCTION:

Disinfectants are used extensively in healthcare settings, playing an important role in the control of infections. Testing the efficacy of disinfectants is a very important component in hospital infection control, but largely overlooked in many hospitals[1]. Antiseptics are agents that destroy or inhibit the growth of microorganisms in or on living tissue while disinfectants are similar but are used on inanimate objects or surface[2]. These agents such as alcohols, phenols, iodine and chlorine were used extensively in hospitals for infection control and prevention of nosocomial infections[3]. An ideal disinfectant, to overcome the antimicrobial resistant pathogens, should have a broad spectrum of antimicrobial activity[4] and the efficacy of these agents may be affected by pH, detergent base, temperature, organic matter, ionic and type of surfactants[5]. Disinfection in hospital practice is mainly achieved either by surface disinfection (eg. disinfection of surfaces of the tables, trolleys, instruments, walls and floors, etc.) or immersing the contaminated objects in the disinfectant solution. Many hospitals are still using phenolic disinfectants, while their use is being discouraged throughout advanced countries[6].

Hospital acquired infections are largely caused by multidrug resistant organisms (MDR) including *Pseudomonas aeruginosa.* These infections can be overcome by proper sterilization and disinfection practices. Various disinfectants are used in the hospitals and healthcare settings; however, their appropriate working concentration is pertinent to achieve disinfection. Hence this study.

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MATERIAL AND METHODS:

This observational study was conducted in the Microbiology Laboratory of NKP Salve Institute of Medical Sciences and Lata Mangeshkar Hospital at Nagpur over a period of 3 months after approval of Institutional Ethics Committee. We tested four disinfectants commonly used in this hospital, namely- Lysol, Bacillocid, Sodium Hypochlorite and Glutaraldehyde by standard methods which were Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test. The organism used for testing was MDR *P. aeruginosa* strain which was also used as a control for the testing methods. No inclusion or exclusion criteria were envisaged (Table 1).

Disinfectant samples were collected in their working concentrations from various Wards,

Table 1: Disinfectants used for testing with their compositions and working concentrations.

Sr. No	Name of disinfectant	Composition	Working Concentration
1.	Bacillocid	Ethylenedioxydimethanol and glutaraldehyde	1% solution
2.	Sodium hypochlorite	Sodium hypochlorite solution	1% solution
3.	Cidex (Glutaraldehyde)	Activated glutaraldehyde Solution	2.2-2.7% solution
4.	Lysol	p-chloro-o-benzyl phenol, ethanol, isopropyl alcohol, potassium hydroxide and hydrogen peroxide (H2O2)	0.5% solution

Intensive Care Units, Operation Theatres, Labor Rooms and OPDs where they are frequently used for disinfection purposes. All these tests for each disinfectant and observed their efficacy. For Rideal-Walker Test (for phenolic disinfectants only)[7], calculated as ratio of 'highest dilution of Lysol that killed in 10 min but not in 5 min' and that of 'phenol that killed in 10 min but not in 5 min', a 24 hour broth culture of *P. aeruginosa* was prepared by inoculating in nutrient broth and incubating at 37°C overnight. Phenol was diluted using distilled water. Five dilutions ranging from 1:95, 1:100, 1:105, 1:110 and 1:115 were prepared and 5 ml of these dilutions were taken separately in five different test tubes and labeled according to their corresponding dilution. In each test tube, 0.2 ml of *P.aeruginosa* broth was added and the test tube was shaken thoroughly. From these test tubes of phenol dilution and culture mixture, subcultures were made into 5 ml of nutrient broth at time intervals of 2.5, 5, 7.5 and 10 minutes for each dilution and labeled accordingly. These test tubes were incubated at 37°C for a period of 72 hours. The tubes were then observed for presence or absence of turbidity. The whole procedure was repeated with lysol using dilutions 1:100, 1:200, 1:250, 1:300 and 1:350 and results were compared with that of phenol. The Rideal-Walker coefficient was calculated by dividing the highest dilutionof lysol that killed in 10 min and not in 5min by highest dilution of phenolthat killed in 10 min but not in 5 min. To perform Chick-Martin Test (for phenolic disinfectants only)[8], a small quantity of about 3 ml of autoclaved yeast suspension was prepared and dissolved in 100 ml of distilled water to give a 3% v/v suspension. To 48 ml of this yeast suspension, 2 ml of 24 hour broth culture of *P. aeruginosa* was added. Lysol was diluted serially from 1:100, 1:200, 1:250,1:300 up to 1:350 and phenol was diluted from 1:95, 1:100, 1:105, 1:110 and 1:115. A 2.5 ml of Lysol dilution was mixed separately with 2.5 ml of the culture-yeast suspension. Lysol was made to act in the presence of yeast suspension to simulate the presence of organic matter. After a contact time of 30 minutes, a standard loop full of the lysol- *P. aeruginosa*- yeast suspension was transferred in duplicate to 10 ml of nutrient broth and incubated at 37°C for 48 hours; thereafter the presence or absence of growth was recorded. The Chick-Martin coefficient was calculated by dividing the concentration of phenol by the concentration of lysol at which similar presence or absence of growth was recorded.

Kelsey-Sykes Test[9,10] was conducted to determine concentrations of disinfectant that will be effective in clean and dirty conditions. The disinfectant was challenged by three successive additions of bacterial suspension during the course of the test. The duration of the test took over 30 minutes to perform[11,12]. In 'clean condition', a 24 hour broth culture of *P. aeruginosa* was prepared and was added to 3 ml of each disinfectant (Bacillocid, Sodium hypochlorite and Glutaraldehyde) respectively having concentration of 1% at time intervals of 0 minutes, 10 minutes and 20 minutes. After a contact time of 8 to 10 minutes, 0.2 ml of the disinfectant- *P.aeruginosa* mixture was transferred to 9 ml of sterile peptone broth containing Tween 20 in three sets of five replicates at time intervals of 8 minutes, 18 minutes and 28 minutes and labeled accordingly. All inoculated peptone broth tubes were incubated at 37°C for 48 hours after which the tubes were examined for growth (turbidity) or no growth. This procedure was repeated for all disinfectants at concentrations of 0.5% and 0.25% using the same time intervals of contact. In 'dirty condition', the procedure was same as for clean condition along with addition of autoclaved yeast cells to 3 ml of each of the disinfectants under test at concentrations of 1%, 0.5% and 0.25% with same time interval of contact.

Two types of surfaces were chosen- smooth and rough for Surface Disinfection Test[13,14] and the efficacy of the disinfectants on these surfaces was evaluated. For 'smooth surface', a 24 hour broth culture of *P. aeruginosa* was prepared and its turbidity was adjusted to 0.5 McFarland standard which is equal to 1.5×10^8 colonies per ml in normal saline. Four glass

slides of dimensions 7 cm x 3 cm were autoclaved out of which three slides were used for testing of each disinfectant (Bacillocid, Sodium hypochlorite and Glutaral-dehyde) and one slide was taken as a control and was labeled accordingly. Further, 0.25 ml of microbial suspension of *P. aeruginosa* was evenly spread over each of the labeled slides with a micropipette and was allowed to dry for 1 to 2 hours. To the three labeled slides, their corresponding disinfectants were applied by a sterile cotton gauge soaked in 5 ml of the respective disinfectant. One slide was left without disinfectant which was used as the control showing only the growth of the test organism i.e. *P. aeruginosa.* After a contact time of 10 to 15 minutes, all the four slides were swabbed using sterile swab sticks and each swab stick was vortexed in four tubes containing 5 ml of neutralizing broth. Serial dilutions of ranging from 1:10, 1:100 and 1:1000 of this mixture were made for each disinfectant and for the control. Five drops of 0.2 ml of each of the dilutions were dropped on Mueller Hinton agar plates and labeled specifically. This was performed for all the disinfectants as well as for the control. These agar plates were incubated for 48 hours for bacterial growth at 37°C and for 7 days at 22°C. After incubation, the number of colonies of microorganisms was counted on control and test slides, total counts were calculated and log reduction factor was calculated. For 'rough surface', while using same procedure, ceramic tiles of dimensions 5 cm x 5 cm were taken in place of glass slides. After incubation, the number of colonies of microorganisms was counted and total counts were calculated. For analysis of surface disinfection test, an average of multiple observations was taken and $\log_{10}$ reduction factor was calculated by using the following formula: Log_{10}Reduction Factor (RF) =

$$\text{Log}_{10}\text{Prevalue} - \text{Log}_{10}\text{Postvalue}.$$

To conduct 'In Use Test', a 1 ml sample of the disinfectant in its working concentration was added to 9 ml of diluent which was taken to be normal saline. Ten drops, each of 0.02 ml volume of the diluted sample are placed on each of the two nutrient agar plates. One is incubated at 37°C for three days and the other at room temperature for seven days. This procedure was performed for all the three disinfectants namely Bacillocid, sodium hypochlorite and glutaraldehyde. Five or more colonies on either plates indicated contamination.

All the above mentioned tests for evaluating efficacy of disinfectants were done in controlled and sterile conditions using the recommended working concentrations of each disinfectant. Accordingly, for each test, the results were calculated and statistically analyzed using standard statistical tests.

RESULTS:

At 1:100 and 1:200 dilutions of Lysol, growth was recorded at 2.5 minutes but not at 5 minutes and above for both dilutions Rideal Walker Test (Table 2). At dilution of 1:250 of Lysol, growth was observed at 2.5 and 5 minutes' contact time, but not at 7.5 and 10 minutes' contact time. At 1:95 dilution, growth was recorded at 2.5 minutes but not at 5 minutes and above. At dilution of 1:100 of Phenol, growth was observed at 2.5 and 5 minutes' contact time, but not at 7.5 and 10 minutes' contact time. Thus, giving a Rideal-Walker coefficient of 2.5. Chick-Martin coefficient, the ratio of 'concentration of phenol in which growth was seen' and 'concentration of Lysol in which growth was seen', was found to be 0.95/0.4= 2.3 by Chick Martin Test (Table 3). "+" denotes growth in the recovery medium and "-" denotes no growth in the recovery medium.

Table 2: Determination of Rideal-Walker coefficient for Lysol and Phenol using *Pseudomonas aeruginosa*as test organism.

Disinfectant	Dilution of Disinfectant	Contact time with culture (in minutes)			
		2.5	5	7.5	10
Lysol	1:100	+	-	-	-
	1:200	+	-	-	-
	1:250	+	-	-	-
	1:300	+	+	+	+
	1:350	+	+	+	-
Phenol	1:95	+	-	-	-
	1:100	+	+	-	-
	1:105	+	+	-	-
	1:110	+	+	+	-
	1:115	+	+	+	+

"+" denotes growth in the recovery medium and "-"denotes no growth in the recovery medium.

Rideal-Walker coefficient = Highest dilution of Lysol that show growth in 5 min but not in10 min / Highest dilution of Phenol that show growth in 5 min but not in 10 min

The Rideal-Walker coefficient was found to be 250/100 = 2.5

Table 3: Determination of Chick-Martin coefficient for Lysol and Phenol.

Disinfectant	Concentration (%)	Subcultures	
		1	2
Lysol	1	-	-
	0.5	-	-
	0.4	+	+
	0.33	+	+
	0.28	+	+
Phenol	1.05	-	-
	1	-	-
	0.95	+	+
	0.90	+	+
	0.87	+	+

"+" denotes growth in the recovery medium and "-" denotes no growth in the recovery medium.
Chick-Martin coefficient =
Concentration of phenol in which growth was obtained
Concentration of Lysol in which growth was obtained

The Chick-Martin coefficient was found to be 0.95/0.4= 2.3.

Table 4: Results of Kelsey-Sykes Test

A. Clean Condition			
Disinfectant	Growth in contact time after subculture		
	8 min	18 min	28 min
Bacillocid	---++	----+	-----
Sodium Hypochlorite	-----	---++	--+++
Glutaraldehyde	----+	---++	-++++
B. Dirty Condition			
Disinfectant	Growth in contact time after subculture		
	8 min	18 min	28 min
Bacillocid	-----	-----	-----
Sodium Hypochlorite	----+	---++	---++
Glutaraldehyde	---++	---++	--+++

At the working concentration of the disinfectant used in both clean and dirty conditions, the results of the growth obtained after subculture showed in Kelsey-Sykes Test (Table 4) that Bacillocid had good antimicrobial activity challenged at time intervals of 8 minutes, 18 minutes and 28 minutes as compared tothat of Sodium hypochlorite and Glutaraldehyde which showed reduced antimicrobial activity as increasing bacterial growth was obtained at increasing time intervals of 18 to 28 minutes. The 'Surface Disinfection Test' done on smooth and rough surfaces in Surface Disinfection Test: (Table 5 and Table 6) showed that Bacillocid was effective on both the surfaces. On statistical analysis, it was seen that p value was 0.8371 using the formula $Log_{10}RF$ *(Reduction Factor)* $= Log_{10}PreV$ *(Pre value)* $- Log_{10}$ *Postv (Post value),* which was not statistically significant. Efficacy of the disinfectants was estimated in In- Use Test: (Table 7) on the principle that five or more colonies on either of the subculture plates indicated contamination. Bacillocid had good efficacy at both room temperature and at 37°C in its working concentration.

DISCUSSION:

The efficacy of four disinfectants was compared by different standard methods like Rideal-Walker Test, Chick-Martin Test, Kelsey-Sykes Test, Surface Disinfection Test and In-Use Test.

The Rideal-Walker coefficient in the present study study was 2.5. In a study by Elias *et al.* in 2013, they studied efficacy of disinfectants such as Izal and Dettol with phenol, and got a Rideal-Walker coefficient of 2.5[10]. The present study is comparable with the findings of Elias *et al* of 2013. Phenolic disinfectants that are more effective than phenol have a coefficient more than 1. Those that are less effective have a coefficient less than 1.

In the study, Chick-Martin test was performed where yeast cells were used and *P. aeruginosa* which acted as a test organism. We found that the Chick-Martin coefficient was 2.3. However, in a study by Elias *et al.* in 2013, they found the Chick-Martin coefficient for Izal and Dettol when compared to phenol to be 2.

Kelsey-Sykes test showed that Bacillocid achieved 0% culture positivity after disinfection or 100% high level disinfection compared to Glutaraldehyde and Sodium hypochlorite. The culture positivity in clean and dirty conditions was determined. The findings of the study are comparable with a study by Malkit Singh *et al(2012)* who found bacillocid to be a very effective antimicrobial agent on both the test conditions [13].

In the present study, an attempt was made to evaluate the efficacy of the commonly used disinfectants in a healthcare setup by different standard methods. Awodele *et al 2007* from Nigeria did a study using similar organisms and methylated spirit, sodium hypochlorite, savlon, kerosene as disinfectants and revealed that savlon was a 100% effective microbicide[15]. Miles R Majcher *et al* 2008 from Canada studied sporicidal activity of sodium hypochlorite, accelerate hydrogen peroxide, chlorine

Table 5: Results of surface disinfection test on smooth surface

Concentration	Disinfectant	Log10 Reduction Factor	Efficacy
1:10	Glutaraldehyde	0.6353	Less effective
	Sodium Hypochlorite	0.6540	Less effective
	Bacillocid	0.9489	Effective
1:100	Glutaraldehyde	0.0366	Less effective
	Sodium hypochlorite	0.0757	Less effective
	Bacillocid	0.0910	Effective
1:1000	Glutaraldehyde	0.0191	Less effective
	Sodium hypochlorite	0.0617	Less effective
	Bacillocid	0.0793	Effective

Table 6: Results of Surface disinfection test on rough surface

Concentration	Disinfectant	Log10 Reduction Factor	Efficacy
1:10	Glutaraldehyde	0.9617	Less effective
	Sodium hypochlorite	0.8353	Less effective
	Bacillocid	1.0136	Effective
1:100	Glutaraldehyde	0.0275	Less effective
	Sodium hypochlorite	0.0139	Less effective
	Bacillocid	0.6504	Effective
1:1000	Glutaraldehyde	0.0234	Less effective
	Sodiumh ypochlorite	0.0101	Less effective
	Bacillocid	0.0495	Effective

Table 7: Results of In-Use test.

Disinfectant	Subcultures (No. of colonies)		Efficacy
	1 (Room temp.)	2 (At 37°C)	
Control	>5 colonies	>5 colonies	-
Glutaraldehyde	>5 colonies	>5 colonies	Not effective
Sodium hypochlorite	>5 colonies	>5 colonies	Not effective
Bacillocid	3 to 4 colonies	No growth	Effective

dioxide, peracetic acid and found peracetic acid acting fastest followed by hypochlorite and accelerated hydrogen peroxide[16]. In the study, when microbicidal activity was studied for four different disinfectants by standard methods, bacillocid was shown to have good antimicrobial activity at a given working concentration and specific contact time.

The effective use of disinfectants constitutes an important factor in preventing hospital acquired infections. The organism tested was known to be a common contaminant and colonizer of patients, Intensive Care Units, Operation Theatres, laboratory surfaces, etc. In the study, *P. aeruginosa* was the most common pathogen in the hospital setup. Hence, we used this as a test organism. Among all the tests employed for testing efficacy of disinfectants, we found that Bacillocid was more effective than the other three disinfectants studied.

There were times when no disinfection policies were in place for the use. The situation has changed markedly in the recent era and many hospitals do have such policies, but implementation is unsatisfactory. The study suggests the need for strict vigilance by the authorities over the local products. Also, there is a need to test the quality of disinfectants routinely supplied to the laboratory or hospital to ensure proper control of infections by using the right disinfectant in right concentration for a right contact time. In all the tests performed for evaluating the efficacy of disinfectants. It was seen that bacillocid was more effective than sodium hypochlorite and glutaraldehyde when tested under different conditions and in the working concentration in which they were used in the hospitals.

In Rideal-Walker test, phenolic agents were tested for their efficacy. Although phenolic agents exhibit high toxicity and low biodegradability, those are still in use in developing countries because of their low cost. Phenolic agents showed poor activity as microbicides. Phenolic agents cannot be used in neonatal and pediatric ICU as they cause eye irritation, contact dermatitis/ urticarial and depigmentation of the skin.[17,18] In this study, phenolics showed poor activity, therefore, better and safer disinfectants are required to replace them. Capacity test of Kelsey-Sykes evaluates the efficacy of disinfectant in clean and dirty conditions. The disinfectants were subjected to a triple challenge test along with the test organism, each consisting of five subcultures. The disinfectant that showed minimal or no growth in the subcultures at specific contact time intervals proved to be more effective than the others. Bacillocid proved to be an effective disinfection agent in comparison with that of glutaraldehyde and sodium hypochlorite. In surface disinfection test, p value was statistically not significant. Bacillocid was found to be an effective antimicrobial agent on the tested organism and on both types of surfaces. In-use test helped to evaluate the disinfectant efficacy used in its working concentration. In the study, Bacillocid passed the test and showed high antimicrobial activity.

CONCLUSION:

Although significant progress has been made with bacterial investigations, the mode of action of

antiseptics and disinfectants still remains to be deciphered. It will also make for efficient use of these agents clinically with the potential for design of newer, more effective compounds and products. An ideal disinfectant, being easily available, non-toxic, non-corrosive, easy to use and cost effective, is difficult to find. Hence, there is need for formulation, implementation and supervision of a comprehensive disinfection policy in the country.

LIMITATIONS OF THE STUDY:

The tests for anti-mycobacterial and antiviral effects of any of the test disinfectants were not conducted. In addition, each test performed here for efficacy testing of disinfectants has its own limitations. Those are cumbersome and complex and Efficacy of locally available disinfectants largely depends on manufacturers' instructions.

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Etiological Factors, Clinico-Hematological Profile and Severity of Anaemia: A Hospital Based Study

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Original Research Article

ABSTRACT

This hospital based cross-sectional study was undertaken to assess etiological factors, clinico-hematological profile and severity of anaemia among patients above 18 years of age in a tertiary care hospital in Bhopal. Consecutive patients diagnosed with anemia based on WHO classification were recruited from out patients and from admitted patients in wards of Department of Medicine, PCMS, Bhopal. A total of 142 patients aged between 18-83 years were recruited in the study. The mean age of the participants was 31.8 years and majority (64%) were females. The commonestpresenting clinical feature was pallor (68%). The mean hemoglobin among the participants was 7.2 gm/dl. The mean MCV, MCH and MCHC were 72.7 fl, 24.8 pg and 27.3 gm/dl, respectively. Most(60%) patients had a peripheral smear picture of microcytic hypochromic anemia. The etiology was: iron deficiency anemia 75%, anemia with chronic diseases 14%, hemolytic anemia 4%,anemia associated with acute illness 4%. Pancytopenia was diagnosed in 11% and megaloblastic anemia in 5%. Severe anemia was diagnosed in 59% of patients. Iron deficiency is the most common cause of anemia in thestudy. Females are at more risk for severe anemia. Prevention and intervention programs for severe anemia are required with main focus on females.

KEY WORDS: anemia, iron deficiency, microcytic anemia, nutritional anemia

INTRODUCTION:

Anemia is a major public health problem, especially in low and middle-income countries like India, regardless of the fact that this problem is largely preventable and easily treatable. According to the World Health Organization (WHO), anemia is defined as hemoglobin (Hb) levels <12.0 g/dL in women and <13.0 g/dL in men. However, normal Hb distribution varies not only with sex but also with ethnicity and physiological status. New lower limits of normal Hb values have been proposed, according to ethnicity, gender, and age. Anemia is often multifactorial and is not an independent phenomenon[1]. The causes of anemia include iron and other micronutrient deficiencies, acute and chronic infections, inherited or acquired disorders, etc[2]. Patients with anemia present with similar clinical symptoms such as fatigue, breathlessness, and dizziness[3]. Anemia also increases the susceptibility to different kinds of infections and impairs the work capacity[4]. Severity of symptoms caused by anemia is paralleled with the severity and speed of development ofanemia[5]. Severe anemia may predispose to infection and heart failure[6].

Most common type of anemia in resource-poor settings is nutritional anemia. Nutritional anemia can be due to iron deficiency (most common cause), folic acid deficiency, vitamin B12 deficiency or may be combination of these factors, which can present with dimorphic picture[7]. These conditions are seen in all types of medical practice ranging from neonatology to geriatrics and public health and are an ongoing concern to all physicians. Other types of anemiainclude hemolyticanemia, which can be either congenital or acquired, aplastic anemia, anemia due to blood loss and anemia of chronic disease[7,8]. Congenital causes include membrane defect, hemoglobin defects and enzyme defect while acquired causes can be immune or non- immune. are the some other types of anemia[7,8].

The present study was undertaken to determine the etiological factors, clinical features, types and grades of anemia in a heterogeneous adult population of the out patient clinics and among admitted patients of Department of Medicine, Peoples College of Medical Science(PCMS), Bhopal.

MATERIAL AND METHODS:

This was anobservational cross-sectional

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study conducted between January 2018 and May 2018. The study participates were consecutive patients aged above 18 years, seeking healthcare in either the out patient clinics or from the consecutive admitted patients in the wards of Department of Medicine, PCMS, Bhopal.

The inclusion criteria were: 1) Patients of age 18 years and above; 2) Patients with symptoms, signs of anemia and diagnosed anemia based on WHO criteria. The exclusion criteria were: 1) Anemia due to acute blood loss; 2) Pregnant women.

Sample size calculation was done based on the review of haemoglobin values for one month in outpatients and among the admitted patients. This chart review provided the prevalence of all cause anaemia as 50%. Thus, to detect at least a 10% difference around the proportion of 0.5 with power of 90%, two-sided alpha of 0.05, the minimum sample size required was 113 patients.

A pretested structured questionnaire was used for this study. A detailed history, and thorough clinical examination was done for all included patients in the study and was recorded in the questionnaire. Written informed consent was obtained from all the participants. WHO criteria for anemia was used for the diagnosis and WHO grades of severity was used grading. All the patients included in the study, underwent following investigations: Haemoglobin, RBC count, leukocyte count, platelets count, PCV (Hematocrit), reticulocyte count, MCV, MCH, MCHC, Peripheral smear examination red cell morphology, haemoglobin electrophoresis, test for sickling, G-6-PD deficiency test.

Data was collected in had copies and were entered in EPI data software and transferred to Stata. Analysis was done using Stata 13 (Stata College Station, TX: Stata Corp LP). The study was conducted in accordance with the Declaration of Helsinki, and the study was approved by institutional ethics committee.

RESULTS:

The study included 142 patients. The age of the study participates was between 18 to 84 years. The majority of patients were from age group 18-35 years 55% (n= 78). The mean age of the study participants was 31.8 years. Majority of participants were females 64% (n=91). The common presenting clinical featureswere – pallor 68%(n=96), weakness 54% (n=76), fatigue 32% (n=46), pain in abdomen 27% (n=39), fever 22% (n=31), reduced appetite 18% (n=26) swelling 18% (n=25) and breathlessness 12% (n=17).The commonest etiological factor leading to anemia wasiron deficiency anemia 75% (n=107), followed by anemia due to chronic diseases 14% (n=20), and acute illnesses 4% (n=6), and hemolytic anemia 4% (n=5) and megaloblastic anemia 3% (n=4). The acute infections included: urinary tract infections (n=2), pulmonary edema (n=1), upper respiratory tract infections (n=1), severe-sepsis (n=1) and pneumonia (n=1). The causes of hemolytic anemia were: a) congenital causes: sickle cell anemia (n=2) and thalessemia (n=2) and b) acquired cause: malaria (n=1). The comorbid diseases included: hypothyroidism (n=7); type 2 diabetes mellitus (n=3), chronic liver diseases (n=3), renal disorders with hypertension (n=5), inflammatory bowel disease (n=1), and chronic obstructive pulmonary diseases (n=1).The mean hemoglobin among the study participants was 7.2 gm/dl. The mean MCV, MCH and MCHC 72.7 fl, 24.8 pg and 27.3 gm/dl, respectively. A total of 60% of patients had microcytic hypochromic anemia and 25% had normocytic normochromic anemia. Pancytopenia was diagnosed in 11% of the patients and 5% had megaloblastic anemia. Severe anemia was found in 59% (n=84) of patients and moderate anemia in 39% (n=55).

Table 1: Distribution of anemia according to gender among enrolled participants.

Variable	N=142	Percentage
Sex		
Male	51	36%
Female	91	64%

Table 2: Distribution of anemia according to clinical presentation among enrolled participants.

Clinical presentation	N=142	Percentage
Fever	31	22%
Reduced appetite	26	18%
Weakness	76	54%
Fatigue	46	32%
Dizziness	16	11%
Pain in abdomen	39	27%
Pallor	96	68%
Jaundice	11	8%
Swelling	25	18%
Breathlessness	17	12%

DISCUSSION:

Anemia due to iron deficiency is the most widespread disease globally. About 50 per cent of women of reproductive age and 26 per cent of men in the age group of 15-59 years are anemic (ACC / SCN, 1987 and Beard, 2005). The findings are in line with the present study i.e. 55% patients in present study were belonging to 18-35 years of age. The complica-

Table 3: Distribution of anemia according to etiology among enrolled participants.

Diagnoses	N=142	Percentage
Iron deficiency anemia	107	75
Anemia due to chronic disease	20	14
Anemia due to acute disease	6	4
Hemolytic anemia	5	4
Megaloblastic anemia	4	3

Table 4: Distribution of anemia according to WHO grade for anemia among enrolled participants.

Grades of anemia	N=142	Percentage
Mild anemia	3	2
Moderate anemia	55	39
Severe anemia	84	59

Table 5: Classification of anemia according to peripheral smearexamination among enrolled participants.

Peripheral smear findings	N=142	Percentage
Microcytic hypochromic	85	60
Normocytic normochromic	35	25
Megaloblastic	7	5
Pancytopenia	15	11

-tions of severe anemia include compromising the work performance, reduction with immune competence and increasing resistance to infection (ACC/ SCN, 1987). The main burden of anemia is on women of reproductive age group. The present study also emphasizes the same as 64% of participants were females with a age group of 18 to 35 years. Similar findings were reported in a study done in Iran by Sadeghian et al[10]. The preponderance of the anemia in the reproductive age group suggests multi factorial etiology in women. As the pregnant women were excluded from the study, the other factors which, precipitate anemia are nutritional factors leading to iron deficiency anemia (75%), chronic (14%) and acute illnesses (4%). In present study the hypothyrodisum (n=7), type 2 DM (n=3) chronic liver diseases (n=3) renal disorders (n=5) inflammatory bowel disease (n=1) and COPD (n=1).

Anemia of chronic disease is the second most common form of anemia, after iron deficiency anemia [11]. All chronic infections can cause anemia[12]. The high prevalence of infectious diseases worldwide makes this the most common form of anemia after nutritional iron deficiency anemia. This type of anemia is particularly associated with infections accompanied by significant inflammatory features.

The other etiological factors in the present study were acute illnesses i.e. 4% like UTI, pulmonary edema, URTI, severe-sepsis and pneumonia.

Hemolytic anemia constituted 4%, which included sickle cell anemia, halassemia and malaria.

The common clinical features with which participants presented were – pallor 68% (n=96), weakness 54% (n=76), fatigue 32% (n=46) , pain in abdomen 27% (n=39), fever 22% (n=31), reduced appetite 18% (n=26) swelling 18% (n=25) and breathlessness 12% (n=17). The commonest presentation was pallor.

In a study done in Sewagram India by Kalantri et al found that the tongue pallor outperformed other pallor sites and was also the best discriminator of anaemia at haemoglobin thresholds of 7 g/dL and 9 g/dL (area under the receiver operating characteristic curves (ROC area = 0.84 [0.77, 0.90] and 0.71[0.64, 0.76]) respetively[13].

Microcytic hypochromic picture was most common finding on peripheral smear examination (60%). Similar findings were seen in a study conducted at L N Medical College, Bhopal by Ratre et al[14]. Normocytic normochromic anemia was found in 25% of patients in present study. However, in the study done by Ratre et al only 6% patients had Normocytic normochromic anemia. Pancytopenia was found among 11%, which is contrary to study done by Ratre et al (2%)[14].

Megaloblastic anemia was found in 5% of patients in present study which suggests dietary deficiency of folic acid and cobalmine. In our study severe anemia was found in 59% of the cases followed by moderate anemia, which was found in 39% of the patients. On the contrary Ratre et al found moderate anemia as the commonest grade followed by severe anemia 57% vs 41% respectively.

CONCLUSION:

Iron deficiency anemia was the commonest etiological factor in the present study with Microcytic hypochromic picture in the peripheral picture and values of MCV, MCH and MCHC was found in 64% of non pregnant females. This requires early diagnosis, treatment and preventive measures to be taken amongst women of reproductive age group. More attention is needed not only in dietary supplementation but also supplementation in the form of ferrous sulphate tablets to these women.

LIMITATION:

Further investigations in the form of serum iron studies, , bone marrow examination, stool for ova and cyst, stool for occult blood, , serum vitamin B12 level, serum folic acid level, Schilling test for

absorption of vitamin B12 are required but were not done in present study because of financial constraints.

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Sputum Bacterial Spectrum and Predominant Inflammatory Cells in Acute Exacerbations of COPD

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Original Research Article

ABSTRACT

This study was done to observe variation with seasons in sputum bacterial profile and predominant inflammatory cells in patients with AECOPD. Hundred sputum samples were cultured for bacteria and examined for type of inflammatory cells. Predominant bacteria and inflammatory cells and their variation with seasons were noted. Thirty six percent of sputum samples had bacterial growth. Bacterial growth was higher in summer and monsoon (43.3%) than in post- monsoon and winter period (21.2%) (p=0.031). Overall, Pseudomonas aeruginosa was the commonest organism cultured. In summer and post-monsoon, the commonest bacterium was Pseudomonas aeruginosa (16.6% and 15.4% respectively) and in monsoon, it was Klebsiella species (19.3%). Sputum neutrophilia (N>61%) was seen in 91% and sputum eosinophilia (E>3%) in 41% of the samples. There was no significant difference in the predominant inflammatory cells (N and N+ E) in sputum with seasons. Isolated sputum eosinophilia was higher in post- monsoon and winter than in summer and monsoon (12.1% v/s 7.5%, p=0.021). Length of hospital stay was less in patients with sputum eosinophilia than in patients with sputum neutrophilia (9.11v/s 10.12 days, p=0.023). Sputum neutrophilia was associated with higher sputum bacterial isolation. Eosinophilia in the sputum was likely to be associated with a sterile sputum. Bacterial isolation was higher in summer and monsoon than post- monsoon and winter. There was no significant difference in the predominant inflammatory cells with seasons. Sputum eosinophilia was associated with faster recovery from AECOPD than sputum neutrophilia.

KEY WORDS: acute exacerbations of COPD (AECOPD), bacterial growth, inflammatory cells, sputum eosinophilia, sputum neutrophilia, sterile sputum

INTRODUCTION:

Acute exacerbations of COPD (AECOPD) contribute significantly to morbidity and mortality. Pathogen infection-related inflammation is the major cause of AECOPD[1].

Both viruses and bacteria, either independently or in combination have been implicated in exacerbations. Bacteria causing AECOPD vary between geographical areas. Studies conducted in various countries showed the predominance of Streptococcus pneumonae, followed by Haemophillus influenzae, Moraxella catarrhalis, Stayphylococcus aureus and other gram negative bacteria whilst those in India showed predominance either of Streptococcus pneumonae or gram-negative bacteria eg. Pseudomonas aeruginosa, Klebsiella and E.coli [2-8].

Although COPD exacerbations are typically associated with increased neutrophilic inflammation, alterations in lower airway inflammation during exacerbations is not completely understood. Neutrophil predominance has been seen in sputum of patients with AECOPD caused by bacteria and eosinophil predominance in viral exacerbations[9-12].

The type of lower airway inflammation may relate to treatment response in AECOPD. Sputum eosinophilia is associated with corticosteroid responsiveness, whereas high bacterial load and sputum purulence with antibiotics[11-13].

This study was conducted to see the variation with seasons in bacterial profile and predominant inflammatory cells in sputum of patients with AECOPD in central India.

MATERIAL AND METHODS:

This was an observational (prospective, cross-sectional) study with a sample size of one

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hundred. The study was conducted after obtaining ethical clearance from Institutional Ethical Committee of People's College of Medical Sciences and Research Centre, Bhopal. One hundred and twenty six patients above the age of 40 years were evaluated for the study over a period of one year six months from January 2017 to June 2018. Twelve patients were excluded for not expectorating sputum and fourteen patients for giving inadequate sample.

Twelve patients were readmitted twice and one patient was readmitted thrice in one and half year of study duration. All patients aged 40 years and above, presenting to Pulmonary Medicine ward, and fulfilling the acute exacerbation of COPD diagnostic criteria according to GOLD 2015 were evaluated by medical interview and physical examination[14].

The inclusion criteria were: 1. Pre-diagnosed COPD patients aged > 40 years; 2. Patients aged > 40 years and newly diagnosed AECOPD. The exclusion criteria were: 1. Patients with Obstructive Pulmonary Disease other than COPD; 2. Patients associated with other lung diseases e.g. pneumothorax, lung carcinomas, active PTB, pleural effusion, pulmonary throm-boembolism; 3. Concurrent reason for worsening of COPD symptoms e.g. acute myocardial infarction, congestive cardiac failure; 4. Conditions associated with the inability to produce sputum e.g. dry cough, patients on ventilator support, unconscious patients; 5. Patients who received antibiotics and corticosteroids seven days prior to admission and 6. Uncooperative patients or those not willing to participate.

Medical history was obtained using a questionnaire applied by a single investigator. Smoking history, place of residence either rural or urban and occupation of patients were taken at the time of admission. Seasons of sputum sample collection were classified according to Indian Meteorological Department: Winter- *January to February*, Summer - *March to May*, Monsoon- *June to September*, Post-monsoon- *October to December*[15].

Patients were requested to keep two to three ml. of sputum after coughing, as far as possible prior to starting antibiotics and corticosteroids. The sample was divided into two. One was subjected to Gram's stain and bacterial culture and sensitivity and another to total and differential leukocyte counts. Most purulent part of the sputum was taken for evaluation. Sputum was examined with light microscope in low magnification field[16]. A sputum sample was considered adequate when <10 epithelial cells and >25 leukocytes (pus cells) were present per low magnification field[17]. Gram's stain, culture and antibiotic sensitivity were done according to standard protocol[18-20].

For sputum cell count, mucus clumps were separated from salivary part of expectorate manually with forceps[1]. Sputum was mixed with WBC diluting fluid in a ratio of 1:1. Counting was done on Neuber's hemocytometer in low power field. For differential leukocyte count, smear was prepared from the sputum. After staining with Leishman's stain, counting of different cells (N, L, E, M) was done according to their morphological characteristics in 100 x field[21].

Based on previous published studies, following normal counts of cells in sputum was taken: TLC <5.5million/ml, Neutrophil <61%, Eosinophil <3%, Lymphocyte <4% and Macrophage <80%[1,11,22]. A sputum neutrophil count >61% was considered sputum neutrophilia and sputum eosinophil count >3% as sputum eosinophilia.

Data analysis was done using SPSS (Statistical Package for the Social Sciences) ver. 20. Quantitative data is expressed as mean whereas categorical data is expressed as number and percentage. Student t test and one way ANOVA was used for quantitative data where as chi Square test was used for categorical data. Level of significance was assessed at 5%.

RESULTS:

Hundred sputum samples were examined between January 2017 and June 2018. Twenty four samples were taken from 12 patients who were admitted twice and 3 samples were from one patient who was admitted thrice. Table 1 shows characteristics of patients. Two thirds of the samples were collected in summer (36%) and monsoon (31%) and the rest were collected in post-monsoon (13%) and winter (20%) seasons. Total duration of summer and monsoon was 11 months and of post- monsoon and winter was 7 months. Bacterial growth was present in 36% of the sputum samples.

Table 2 shows higher bacterial growth in summer and monsoon (43.3%) compared to winter and post- monsoon period (21.2%). Table 3 shows that the predominant bacterium isolated was Pseudomonas aeruginosa (14%), followed by Klebsiella species (10%), E. coli (7%) and Citrobacter (2%). The only Gram positive bacterium, Staphylococcus aureus, was grown in 3% of the patients.

Table 1: Characteristics of patients.

	Parameters		No of patients	Percentage
General Characteristics	Age	41 – 50	6	6
		51 – 60	24	24
		61 – 70	48	48
		71 – 80	21	21
		>80	1	1
	Gender	Male	89	89
		Female	11	11
	Residence	Rural	87	87
		Urban	13	13
	Occupation	Farmer	72	72
		Businessman	12	12
		Serviceman	12	12
		Other	4	4
	Smoking status	Current smoker	37	37
		Ex smoker	49	49
		Non smoker	14	14
	Exposure to form of smoke exposed	Bidi	70	70
		Cigarette	16	16
		Biomass	14	14
Sputum characteristics	Season of sputum collection	Summer	36	36
		Monsoon	31	31
		Post-monsoon	13	13
		Winter	20	20
	Growth present	No of patients	36	36
	Sterile	No of patients	64	64
	Cell count	TLC >5.5million/ml	81	81
	Sputum neutrophilia	Neutrophils >61%	91	91
	Sputum Eosinophilia	Eosinophils >3%	41	41

Table 2: Sputum bacterial growth and seasons.

	Total	Summer and Monsoon (11 months)	Post-monsoon and Winter (7 months)	Total	p value
No. of sputum samples analyzed	100	67 (67%)	33 (33%)	100	
No. of sputum samples with bacterial growth	36	29 (43.3%)	7 (21.2%)	36	0.031

Table 3: Sputum samples and bacterial spectrum.

Bacteria	No. of samples	Percentage of samples in which bacterial growth present
Citrobacter	2	5.5
Staph aureus	3	8.4
E coli	7	19.5
Klebsiella species	10	27.8
Pseudomonas aeruginosa	14	38.8
Total	36	36 (100)

Pseudomonas aeruginosa was the most frequently isolated bacterium in summer (16.6%) and post- monsoon (15.4%). In monsoon, it was Klebsiella species (19.3%). No predominance of any bacteria was seen in winter (Figure 1).

Table 4 shows that out of a total of 100 sputum samples 81 showed increased total leukocyte counts. Sputum neutrophilia was seen in 91 samples and

Table 4: Sputum samples with various sputum inflammatory cells.

Sputum with increased cells	No of sputum samples n, (%)	Total
TLC (>5.5 million/ml)	81 (81)	100
N (>61%)	91 (91)	100
E (>3%)	41 (41)	100
L (>4%)	11 (11)	100
M (>80%)	0	0

Table 5: Predominant sputum inflammatory cells according to seasons.

	Summer and Monsoon					Post Monsoon and Winter					
Inflammatory cells	Summer (6months) N=36 n (%)		Monsoon (5months) N=31 n (%)		Total n, (%)	Post- monsoon (3months) N=13 n (%)		Winter (4months) N=20 n (%)		Total n, (%)	p value
N >61%	22	(61.1)	18	(58.1)	40 (59.2)	6	(46.1)	13	(65)	19 (57.6)	0.728
N+E	10	(27.8)	12	(38.7)	22 (32.8)	6	(46.1)	4	(20)	10 (30.3)	0.458
E>3%	4	(11.1)	1	(3.2)	5 (7.5)	1	(7.7)	3	(15)	4 (12.1)	0.021

Table 6: Sputum inflammatory cells and mean hospital LOS of patients.

Increased sputum inflammatory cells	Mean length of hospital stay in no. of days (std deviation)	p value
N	10.12 (1.34)	0.023
E	9.11 (1.45)	

sputum eosinophilia in 41 samples.

Bacterial growth decreased progressively as neutrophils decreased and eosinophils increased in the sputum. Of the 59 neutrophil predominant samples, growth was seen in 25 (42%). Growth was less in samples containing both N+E (28%) and least in samples with predominant E (22%) (Figure 2).

Table 5 shows that there was no difference in the percentage distribution of isolated neutrophils and mixed population (N+E) in sputum with seasons. Percentage of samples with isolated sputum eosinophilia was higher in post-monsoon and winter than in summer and monsoon (p=0.021).

Table 6 shows that patients with predominant eosinophils in their sputum had shorter mean length of hospital stay (9.11 days) as compared to patients with predominant neutrophils in their sputum (10.12 days) (p=0.023).

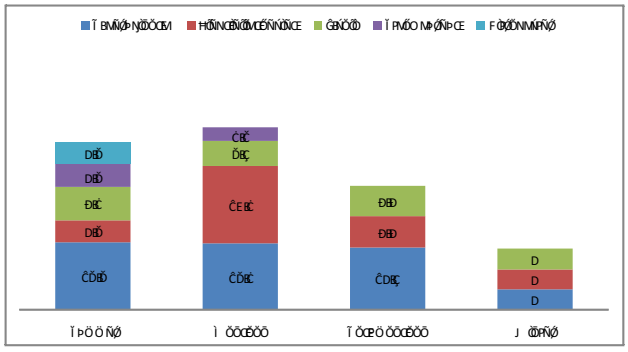

Figure 1: Sputum bacterial spectrum according to seasons.

DISCUSSION:

We did this study to observe the bacterial profile and the predominant inflammatory cell in sputum of patients with AECOPD in various seasons.

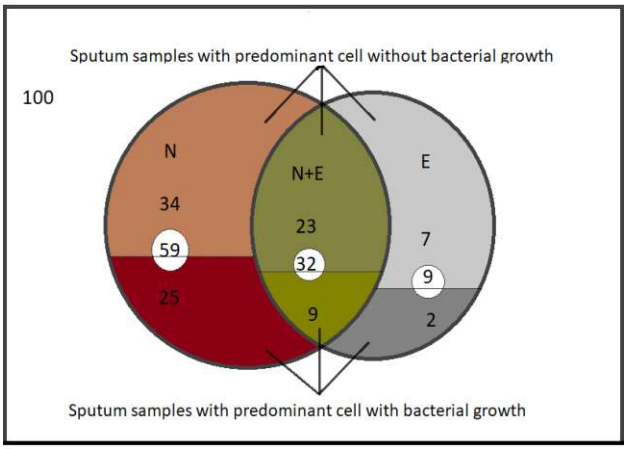

Figure 2: Predominant inflammatory cells and bacterial growth. .

Bacteria causing AECOPD:

In the present study, bacterial growth was present in 36% of sputum samples. This was significantly lower than that reported by Groenewegen et al and Papi et al, who found bacterial growth in 50% to 55% in AECOPD[12,16]. However, Cukic et al reported that growth of pathogenic bacteria was slightly lower, at around 41%[23]. Dai et al observed 21% bacterial growth in their study on patients with AECOPD[24].

According to a systematic review by Uzun and colleagues, bacteria as the cause of AECOPD was reported 30% to 55% cases[25]. Prior use of antibiotics by patients within the past three months could be one of the reasons for culture negativity[26]. According to Kyo et al and Chawla et al, sputum samples collected at times other than morning may not have bacterial growth[27]. In our hospital-based study, patients were admitted any time during day or night and antibiotic treatment was given without any delay in such patients, sometimes before sputum collection.

Collection of morning sputum sample was also not possible every time.

Furthermore, the classical microbial culture techniques using standard conditions can culture only 30% of bacteria[28]. This may explain lower bacterial culture growth of 36% in our study.

The bacterial spectrum varies not only between geographical areas but also, from time to time, within the same geographical area. Studies conducted in various countries showed the predominance of Streptococcus pneumonae followed by Haemophillus influenzae, Moraxella catarrhalis, Staphylococcus aureus and other gram-negative bacteria[2-5]. Studies conducted in India showed predominance either of gram-negative bacteria eg. Pseudomonas aeruginosa, Klebsiella and E.coli or Streptococcus pneumonae[6-8]. In our study, predominance of Gram-negative bacteria was observed. The predominant organism that was isolated was Pseudomonas aeruginosa (14%). Other organisms were Klebsiella species (10%), E.coli (7%) and Citrobacter (2%). The only Gram-positive bacterium, Staphylococcus aureus, was grown in 3% of the sputum samples.

Bacterial spectrum with seasons:

Various cities have reported different hospital admission rates with seasons[29]. In London most of the exacerbations occurred in cold season, November-February[30], whereas in New Delhi, there was no statistically significant difference in admissions per month in winter- *November to February* v/s summer- *March to October*[31].

In our study, admissions with AECOPD were more in summer (36%) and monsoon (31%) than in winter (20%) and post-monsoon (13%). In study period of one and half year, the total duration of summer and monsoon was eleven months and of post-monsoon and winter season was seven months. Bacterial isolation was higher in summer and monsoon (43.3%) than in post-monsoon and winter (21.2%).

The low bacterial isolation in post monsoon and winter could be because of higher viral exacerbations of COPD and/or allergic cause of AECOPD. Previous studies by Donaldson et al and Wedzicha et al suggested that cold and humid environment might favour the occurrence of viral infections[30,32].

Papi et al reported that virus alone was responsible for about 25% of exacerbations of COPD[12]. Almost similar result was reported by Bafadhel et al (29%) in their study[11].

Allergens may increase respiratory symptoms and risk of COPD exacerbation[33,34]. According to Jamieson et al the allergic phenotype accounted for 25% and 30% in two different COPD cohorts and this phenotype was associated with increased risk of COPD exacerbations[35].

The predominant inflammatory cell in sputum in AECOPD:

Majority of the sputum samples (81%) had increased TLC (>5.5 million/ml). COPD exacerbation was associated with increased total leukocyte count in sputum. Nearly all sputum samples, 91%, had neutrophilia >61%. Forty one percent of sputum samples had eosinophilia >3%. Only 11% of the sputum samples had increased lymphocytes >4%. We found that the predominant cell that increased in sputum during exacerbations was neutrophils. Pure neutrophilia was seen in 59% of the samples. Sputum neutrophilia was associated with sputum eosinophilia in 32% of the samples. The two cells that were chiefly seen during exacerbations were neutrophils and eosinophils.

Gao et al observed the predominance of neutrophils, eosinophils and macrophages in AECOPD. Based on the sputum inflammatory cell profile, they classified patients with AECOPD into neutrophilic, eosinophilic, mixed granulocytic and pauci-granulocytic groups[1].

In our study also, exacerbations could be classified as purely neutrophilic (59%), mixed neutrophilic and eosinophilic (32%) and purely eosinophilic (9%) (Figure 2). Papi and colleagues reported that sputum neutrophils were increased in all exacerba-tions of their sixty four patients ($p < 0.001$)[12]. Bafadhel and colleagues found that a substantial number of exacerbations were associated with sputum eosinophilia (28% of 182 exacerbation events) and concluded that eosinophilic airway inflammation also existed in AECOPD[11].

Bacterial growth and predominant sputum inflammatory cells:

Sputum neutrophilia is associated with bacterial etiology of AECOPD[1]. In our study, out of 91 sputum samples which had neutrophilia, 34 (37.4%) had bacterial growth. Sputum neutrophilia was also present in sterile sputum. Papi et al observed that sputum neutrophils were increased in all exacerbation sub-groups: viral, bacterial, viral-bacterial co infection and non infectious[12].

Out of 41 sputum samples which had eosinophilia, bacterial growth was seen in only 11(26.8%) and there was no bacterial growth in 30 (73.2%). Sputum eosinophilia in AECOPD was less likely to be associated with bacterial growth. Similarly, Kolsum et al found that sputum eosinophil percentage was inversely related to bacterial load[10]. Papi and co-workers concluded that sputum eosinophilia was related to viral exacerbations in COPD[12].

Response to therapy and predominant sputum inflammatory cells:

We observed a lesser mean length of hospital stay in patients with sputum eosinophilia as compared to patients with sputum neutrophilia (9.11v/s10.12 days, p=0.023). This could be due to a prompt effect of corticosteroids in patients with allergic phenotype of AECOPD who presented with sputum eosinophilia.

Sputum inflammatory cells according to seasons:

Pure eosinophilic exacerbations were higher in post-monsoon and winter as compared to summer and monsoon (12.1%v/s7.5%, p=0.021). This could indicate an allergic or a viral etiology of exacerbations.

Since sputum eosinophilia was associated with a lesser length of hospital stay, likely due to prompt response to steroid therapy, eosinophilic exacerbations were probably of allergic origin. It would be worthwhile to investigate whether patients with sputum eosinophilia could be treated with systemic steroids without antibiotics.

Although infections are a major cause of exacerbations in COPD, in our study the overall bacterial growth was less (36%) compared to 50-55% in other studies. This could be because many a time sputum samples could not be collected before antibiotic initiation. Patients either were not expectorating sputum at the time of admission or were admitted at night.

Furthermore, sterile sputum could be a result of a viral or an allergic exacerbation. Isolation of virus would have given us an idea as to what proportions of our patients had viral exacerbations. Since patients with sputum eosinophilia had a lower length of hospital stay than those with sputum neutrophilia, it could be interpreted that these patients had an allergic exacerbation that responded promptly to steroid therapy.

To facilitate differential cell count, sputum analysis for inflammatory cells is done after separation of sputum part from salivary part of the expectorate. Clumping of mucus is prevented by adding DTT (Dithiothretal) solution for sputolysis. Finally, the mixture is cytospined[36]. To cut down on the cost of the investigation the sputum in our study was examined after separating the sputum part from the saliva with the help of forceps, without adding DTT solution. This may not prevent the clumping of mucus. Despite this, results of our study are consistent with results of other studies where processing of sputum was done as described above[1,12,36].

CONCLUSIONS:

We have shown that AECOPD is associated with both sputum neutrophilia and sputum eosinophilia. Presence of neutrophils in sputum, in contrast to eosinophils, is more likely to be associated with bacterial exacerbations of COPD. The absence of bacterial growth in samples with eosinophilia could indicate an allergic exacerbation. It would be worthwhile to investigate whether patients with sputum eosinophilia could be treated with systemic steroids without antibiotics.

The principal reason why laboratories do not do sputum cell analysis, is its complexity. We have shown that direct smear method of sputum cell analysis can be done. However, to recommend direct sputum analysis, further studies are required to compare the various techniques of sputum processing.

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Shapiro's Syndrome

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Case Report

ABSTRACT

Shapiro's syndrome(SS) is an extremely rare disease consisting of paroxysmal hypothermia, hyperhidrosis and corpus callosum agenesis[1] with onset typically on adulthood. We reported a case of 15 year old male presented with classical triad of sudden onset of headache associated with episodes of profuse sweating, low body temperature and excessive shaking/shivering. On MR imaging complete agenesis of corpus callosum with colpocephaly, dilated and highly placed 3rd ventricle. Nasal encephalocele and anterior linear falcine lipoma is seen in this case which had not been recorded in previously reported case.

KEY WORDS: colpocephaly, encephalocele, falcine lipoma, shapiro's syndrome

INTRODUCTION:

Shapiro's syndrome is an extremely rare disorder consisting of paroxysmal hypothermia, hyperhidrosis and agenesis of corpus callosum with onset typically on adulthood.[1] It is described by Shapiro and Plum in 1967.[2] There is variation in the frequency and duration of episodes from person to person. Less than 60 cases has been reported till date. The pathophysiology as well as the prognosis of SS are still debatable. Carbamazepine, Clonidine, Valproate Sodium Valproate, propanolol, pizotifen has been used but there is no treatment consensus.

CASE REPORTS:

A 15-year-old male patient presented with complaint of sudden onset of headache associated with episodes of profuse sweating, low body temperature and excessive shaking/shivering lasting for about 15-30 minutes. He also complains of nausea, weakness, vertigo, polydipsia and polyuria. As narrated by patient's mother he developed similar symptoms at the age of 7 years and had similar complaints every 6-8 months for which he was admitted and given symptomatic treatment. The episodes were self resolving and did not have any aggravating factor. There was no history of seizures, confusion and palpitations during the episode. There was no known past history of head or spinal injuries. Family history was not significant.

The patient was admitted in our hospital with episodes of hyperhidrosis, and subsequent hypothermia. On general physical examination a swelling was present over nose and multiple hyperpigmented macules are present over abdomen and trunk (Figure 1). His neurological examination is insignificant. Routine hematological and biochemical investigations were normal. Thyroid hormonal profile, electrocardiogram, X-ray chest and USG abdomen did not reveal any significant abnormality.

MRI brain shows complete agenesis of corpus callosum, Ventricles- run parallel rather than normal bow type configuration giving "racing car" appreance on axial imaging [Figure-2(a)], characteristic "Moose Head or Viking Helmet" appearance on coronal imaging [Figure-2(b)]due to colpocephaly (dilated and widely seperated posterior horn of lateral ventricle)[Figure-2(c)] and dilated and highly placed 3rd ventricle [Figure-2(d)]. Associated with nasal encephalocele[Figure -3(a)] and linear falcine lipoma anteriorly [Figure -3(b)].

DISCUSSION:

The corpus callosum is the largest interhemispheric connective fibre bundle in brain and the largest fibre tract in the cental nervous system[3].The triad of hyperhidrosis, corpus callosum

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Figure 1: Hyperhidrosis with nasal encephalocele and multiple hyperpigmented macules over trunk.

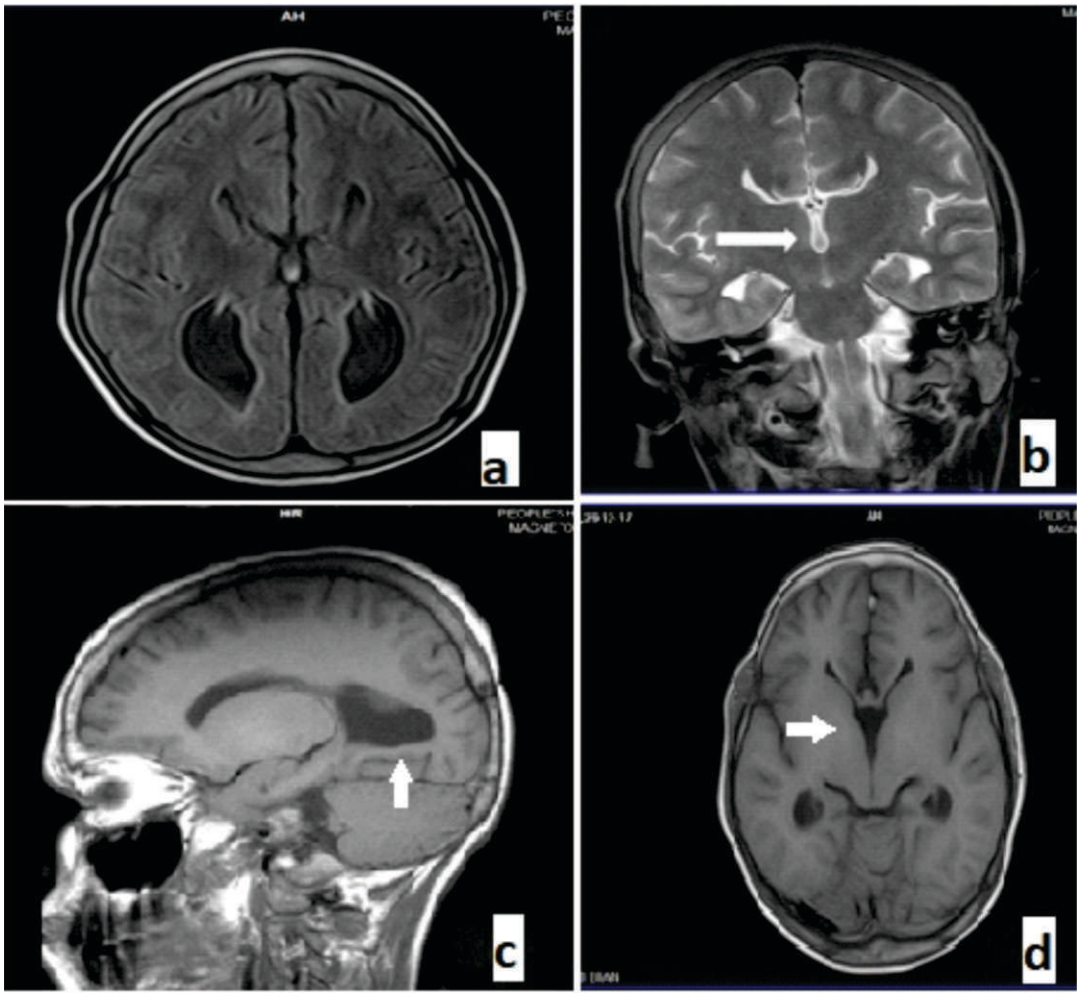

Figure 2: (a) Racing car sign. (b)Moose head or Viking helmet sign. (c) Colpocephaly. (d) highly placed 3rd ventricle.

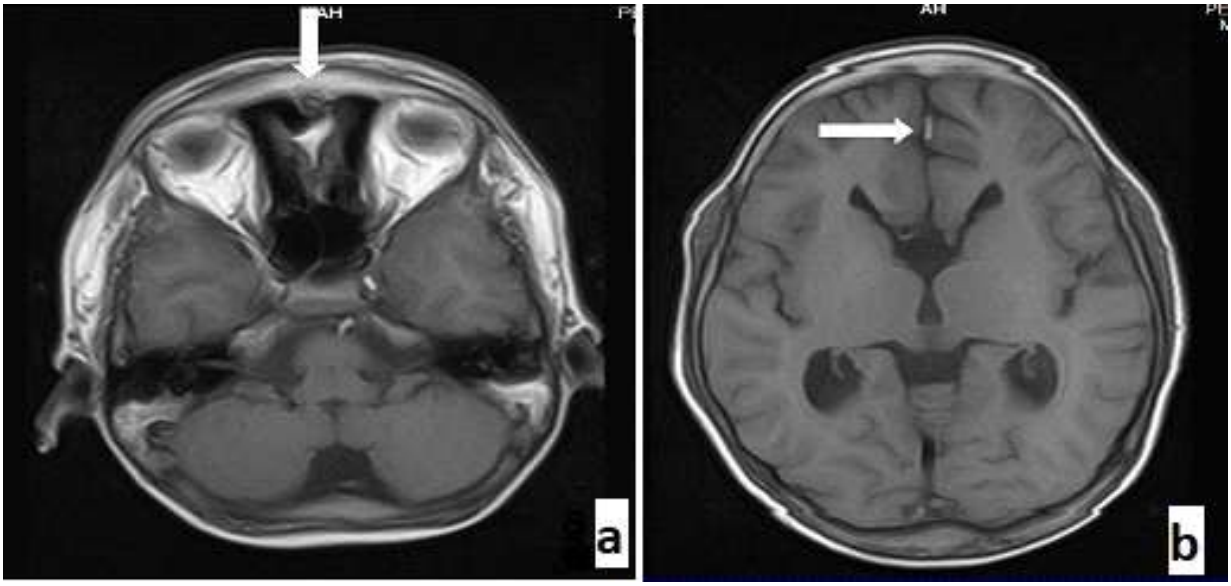

Figure 3: (a)Axial T1WI shows Nasal encephalocele, (b) Falcine lipoma anteriorly on T1WI.

agenesis and spontaneous periodic hypothermia without any hypothalamic lesion pointing towards unusual neurological disorder of SS. It had typical onset in adulthood[3,4]. The duration and frequency of the episodes vary from person to person, with some episodes lasting hours to weeks and occurring from hours to years.[5]

To date, several hypothesis have been suggested regarding the pathophysiologic mechanisms underlying this syndrome. Clearly, the agenesis of the corpus callosum by itself does not cause thermal dysregulation, as callosotomy did not lead to defective thermoregulation and hypothermia .

It is thought that the imbalance between anterior hypothalamic heat-dissipating centre and posterior hypothalamic heat-conserving center causing the fluctuating body temperature in Shapiro syndrome. Suggested mechanisms include central nervous system structural abnormalities, degenerative processes, neurochemical dysfunction, inflammatory processes, and seizure activity.

The differential of the disease are severe hypothyroidism, use of antipsychotic drugs with a strong 5-HT2 antagonistic component and hypoglycemia or attacks of diabetic ketoacidosis [6].

CONCLUSION:

Magnetic Resonance Imaging is the classical imaging tool for the diagnosis of Shapiro syndrome and its variants. There is no cure for Shapiro syndrome. Management is mainly supportive and includes rewarming with warm blanket. We reported this case as its association with nasal encephalocele and anterior linear falcine lipoma which had not been reported till date.

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Corpus Alienum in Orofacial Region: A Diagnostic Challenge

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Case Report

ABSTRACT

In this case report we are presenting an unusual case of foreign body embedment in the oro-facial soft tissue. Our patient is a male adult and presented with history of restricted mouth opening, fever and painful facial swelling in the cheek. Plain radiographs of the maxillo-facial region was not diagnostic hence an ultrasonography was performed and a wooden foreign body which resembled a broken needle was noticed. Ultrasound guided surgical exploration was performed for the retrieval of the foreign body. Prompt diagnosis and early surgical exploration under USG guidance to retrieve such foreign bodies will greatly minimise the morbidity associated with it. This paper highlights the need for clinicians to elicit detailed history, however irrelevant, in arriving at a proper diagnosis, which in turn will influence the diagnosis and extend of surgical exploration.

KEY WORDS: foreign body, orafacial, trismus, ultrasonography

INTRODUCTION:

Penetrating foreign bodies in the Maxillo-Facial region are usually not missed in diagnosis[1]. Foreign body (Latin: corpus alienum) is defined as any microscopic or macroscopic external object which gets introduced into the human body, may be due to some accidental injury or any iatrogenic procedure[2,3].These foreign bodies can be inert or irritating[2] and are made up of metal, wood, plastic or glass[4]. Usually metallic foreign bodies are often inert and may cause no irritation or damage for many years to the surrounding structures but if these foreign bodies irritate, they may develop inflammatory reactions[5], and damage adjacent structures. Hence their diagnosis, identification, location and surgical removal from the tissue are often necessary.

CASE REPORT:

A male patient aged 55 years reported with complaint of swelling and tenderness on left side of cheek since 10 days (Figure 1). Initially, patient did not reveal any significant history prompting radiological investigations. Palpation revealed firm swelling and examination revealed a swelling pointing towards anterior border of masseter and suspected healed punctum. Palpation revealed firm diffused swelling with sharp lacerating pain on pressing on the suspected punctum. A provisional diagnosis of an abscess or sebaceous cyst was arrived at. Intraoral examination and OPG revealed no dental source of infection.

An USG study was ordered to evaluate sebaceous cyst/ Intra muscular lesion. USG revealed (Figure 2) a 2 cm needle like object with lumen within the masseter muscle which was initially suspected as broken needle.

On further enquiry with the patient, he revealed history of fall 6 months earlier on an Acacia bush, where he sustained the prick injury on a thorn, which he claimed to have removed and the pain subsided in few days. Based on the history and USG findings; a diagnosis of wooden foreign body was arrived at. Initial exploratory surgery could not locate the foreign body as the foreign body was entrapped well within the muscle, thereby the patient was shifted to ultrasound room and the foreign body was retrieved (Figure 3) by ultrasonography guided exploratory procedure. Patient was administrated adequate anti-inflammatory and analgesics therapy and the recovery was uneventful.

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Figure 1 : Swelling and tenderness on left side of cheek

Figure 2: HRSG left parotid gland shows a well defined linear hyperechoic foreign body.

Figure 3: Thorn of Acacia bushretrieved surgically.

DISCUSSION:

Corpus alienum or commonly called foreign body is an external object of any shape and size made up of metal, plastic, wood or glass[4]. It can occur in any age with different etiology. The effect of foreign body may differ as per its content and age. Young children usually put objects into their mouth, ears or nose[2]. The emergency of the condition depends on the things they swallow. A coin, usually causes a pressure in tissue and is not a medical emergency but small button, batteries, if ingested, can cause release of hydroxide ions and cause burn and hence becomes a medical emergency to treat[2]. In adulthood, the nature of etiology differs as main cause of foreign body is due to fall, road traffic accident, air gun injury etc. Iatrogenic injuries such as broken needle, sutures, drain and gauze are accidently left over which over a period of time may become source of infection. Though he had history of fall in the bush six months earlier, he thought it was irrelevant and not did inform us. He presented with a classical case of buccal space infection. Hence a provisional diagnosis of sebaceous cyst or buccal space infection was arrived. We conducted radiographic examination but the source of infection remained untraceable. Hence we performed a USG study of left parotid region to rule out cyst, intra muscular lesion or lesion of parotid gland. HRSG revealed a well defined linear hyperechoic body of length 2.3 cm to 3 cm in muscle plane of left cheek region, anterio-posterior to left parotid gland. Since the USG report revealed a linear hyperechoic object with a centre area of liener hypo echoicity, the dilemma was how has a needle penetrated there and how it was missed in the conventional radiograph. Hence, differential diagnosis of foreign body like glass, plastic as well as wooden structure must be kept in mind as these things are not diagnosed by routine conventional radiographs. The oro-facial soft tissue spaces are anatomic spaces filled with loose connective tissue and[2,3] are bounded by bones and muscles[7]. Any foreign body in thin spaces either lie dormant for years or can produce chronic inflammatory reaction and need to be localized and surgically removed[4,5]. In case of foreign body of wooden or plastic origin, USG must be performed prior to manipulation to know the precise location so that the vital structures around it can be preserved. In our patient the wooden spike was present near branches of facial nerve, parotid duct, facial artery and veins. The surgical removal of foreign body can be attempted both under local or general anaesthesia, depend upon its location, medical condition and severity of infection. In our case, as we

located spike in masseter muscles, hence surgical exploration was done under local anaesthesia and none of important anatomical structures were injured.

CONCLUSION:

In our view, a detailed case history, early diagnosis by proper investigations and surgical retrieval are key to prevent complications. Accurate localization of foreign body and knowledge of important structures like nerves, vessels, glands or ducts is necessary to prevent uneventful postoperative recovery.

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Nonketotic Hyperglycemia Induced Dystonia in Middle Age

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Case Report

ABSTRACT

Movement disorders are clinical syndrome with either excess or paucity of voluntary or involuntary movement which is not related to weakness or spasticity (1). Causes of abnormal movements consist of neurological diseases like Parkinson's disease, progressive supranuclear palsy, drug induced, idiopathic and some metabolic causes like uncontrolled blood sugar level. Non-ketotic hyperglycemia (NKH) has been associated with various neurological disorders, and among these, chorea-ballismus is one of the most frequently observed movement disorder. A patient presented to our hospital with involuntary, jerky, unpredictable, non rhythmic, repetitive movements involving left upper and lower limb. On examination his blood sugar level was high with no focal neurological deficit. He was started on insulin therapy. As his blood sugar level came down to normal limits, his movements started disappearing.

KEY WORDS: dystonia, hyperglycemia, non-ketotic hyperglycemia (NKH),

INTRODUCTION:

Nonketotic hyperglycemia, also known as diabetic striatopathy, is a rare cause of involuntary movements as a primary manifestation of diabetes mellitus (2). it mainly affects elderly individuals, presenting as the triad of hemichorea-hemiballism, hyperglycemia, and a lesion in the basal nuclei showing a hyperintense signal on T1-weighted images. Clinical and imaging findings are typically unilateral, although they can be bilateral in up to 11.4% of cases, being potentially reversible.

CASE REPORTS:

A 55-year-old man, with involuntary, jerky, unpredictable, non rhythmic, repetitive movements involving left upper and lower limb. was admitted for movement disorders involving the left side of the body which had appeared acutely 2 weeks previously. He was conscious and alert. Neurological examination revealed choreiform and proximal athetotic movements involving left upper and lower limb. He didn't have any history of focal neurological deficit or preceding trauma or diabetes mellitus and never took medication for hyperglycemia. On examination his blood sugar level was high with no focal neurological deficit.

Laboratory studies demonstrated high glucose values (540 mg/dl) with no evidence of ketosis with normal arterial blood gas with pH (7.44) & HCO3 (22.4 mmol/l). CT-head study shows hyperdense right basal ganglia without mass effect. Patient was initiated with insulin and olanzapine and as blood sugar level came within normal limits, his abnormal movements start disappearing.

DISCUSSION:

Involuntary movements compose a group of uncontrolled movements that may manifest as tremors, tic, myoclonic jerk, chorea, athetosis, dystonia or hemiballismus. These movements can be divided into two broad categories: (a) Hypokinetic-Parkinson's disease, Hallervorden Spatz disease, progressive supranuclear ophthalmoplegia, striatonigral degeneration; (b) Hyperkinetic- dystonia (drug induced or familial), Rheumatic chorea, Huntington's chorea, Tardive dyskinesia, Tourette's syndrome, Tic disorders, Cerebral palsy, and some rare causes like metabolic causes like nonketotic

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Figure 1:Patient showing abnormal position of hand and lower limb due to \abnormal movements.

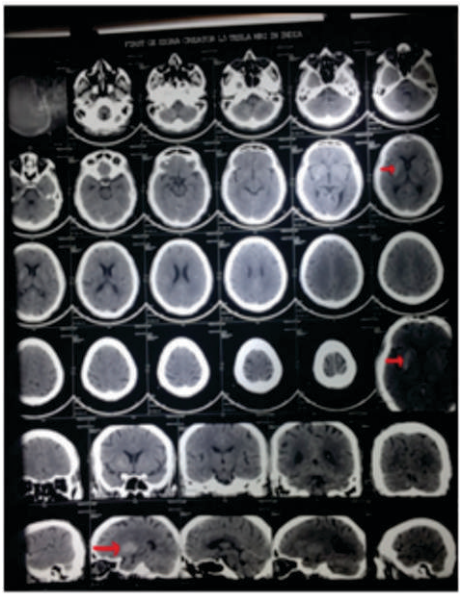

Figure 2:CT head study shows hyperdense right basal ganglia.

hyperglycemia.

Although the pathophysiology of nonketotic hyperglycemia is unknown, some proposed mechanisms include metabolic changes such as the deposition of proteins and degradation products of myelin, blood, calcium, or other minerals, which are likely to decrease as serum glucose level is controlled.

Another accepted theory is that the changes produced by hyperglycemia in perfusion results in reduced Krebs cycle activity, which induces anaerobic metabolism, causing the brain to use alternative sources of energy, and metabolizing the gamma-aminobutyric acid (GABA) inhibitory neurotransmitter. In nonketotic hyperglycemia, GABA and acetate level drops rapidly, leading to decrease in acetylcholine synthesis. It has therefore been speculated that the reduced levels of acetylcholine and GABA in the basal nuclei leads to dysfunction of those nuclei, thus producing involuntary movements such as those seen in chorea-hemiballism(4).

CONCLUSION:

In case of involuntary movements, it becomes necessary to investigate for hyperglycemia for ensuring appropriate and timely treatment. Proper diagnosis of hyperglycemia induced involuntary movements can avoid unnecessary and often ineffective initiation of antiepileptic group of medications or other unwanted medication that can cause economic burden to the patient.

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Betamethasone Induced Hypertrophic Obstructive Cardiomyopathy in Infant

Case Report

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ABSTRACT

Hypertrophic obstructive cardiomyopathy (HOCM) is a major potential complication of dexamethasone therapy in preterm infants. HOCM may also be caused by Betamethasone in infants if given for longer time. We present a case of a 3 month old male baby who was prescribed betamethasone by quack for 1 month for treatment of cough and cold. The baby developed HOCM and systolic anterior motion of mitral valve which completely resolved after stoppage of betamethasone in 12 weeks.

KEY WORDS: betamethasone, hypertrophic cardiomyopathy (HOCM), infant

INTRODUCTION:

Hypertrophic cardiomyopathy (HCM) is a heterogeneous, relatively common, and potentially life-threatening form of cardiomyopathy. The causes of HCM are heterogeneous and include inborn errors of metabolism, neuromuscular disorders, syndromic conditions, and genetic abnormalities of the structural components of the cardiomyocytes[1]. Unfortunately, use (and misuse) of several drugs and medications are well known causes of injury to cardiac muscles[2].

Steroid like dexamethasone, is widely used in cases of chronic lung disease (CLD) for both prevention as well as management in infants. Case reports are published mentioning the use of steroids in decreasing the duration of ventilator dependence. Prolonged use of these steroids leads to development of HCM[3]. When these steroids are stopped, there occurs the reversal of echocardiographic changes over 2-3 weeks[4]. Most of the published reports on drug induced HCM mention dexamethasone as a cause. We present a case report of betamethasone induced HCM which was totally reversed over 3 months.

CASE REPORTS:

A 3 months old male baby presented with tachycardia, tachypnea and fever since 3 days. There was history of 2 episode of upper respiratory tract infection in last 2 months. Patient visited local practioners where he was prescribed oral medications. On examination child was having cushingoid facies and respiratory distress. BP 98/52, HC 39.5 cm, Wt 7 kg (>90th centile), bilateral breath sounds equally heard with course crepitations, systolic murmur, soft abdomen with palpable liver of 4 cm below coastal margins, spleen not palpable. History revealed that the child was prescribed symptomatic treatment, oral antibiotics and oral betamethasone drops by local quacks. Parents also revealed that as the symptoms were frequent therefore they were advised to take oral betamethasone drops daily for 1 month. Baby started gaining weight which parents considered it to be normal. There was no history of previous hospitalisation.

On investigation complete blood picture, liver and kidney functions and serum electrolytes, urine routine microscopy were all within normal limits. Serum cortisol 17.07µg/dL, serum cholesterol 295 mg/dL, ultrasound abdomen and electrocardiograph were within normal limit. Echocardiographic findings were showing concentric left ventricle (LV) hypertrophy (symmetrical), inter ventricular septum (IVS), posterior wall (PW) 10 mm, peak left ventricular outflow tract gradient 24 mmHg, systolic anterior motion of mitral valve, moderate mitral regurgitation with normal biventricular functions. During course of stay patient was managed symptomatically and betamethasone was stopped.

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Patient was discharged after 5 days and kept under follow up. A repeat echocardiography after 12 weeks showed reversal of HCM and normal study (right ventricle 13, aorta 11, left atria 15, PW/IVS 04/03, Left ventricle internal diameter end diastole and end systole were 17 and 14 respectively, ejection fraction 60%).

DISCUSSION:

Steroid-induced hypertrophic cardiomyopathy is a distinct clinical and echocardiographic entity for infants on steroids. Some insights into the underlying mechanisms have been provided by animal experiments[5]. For premature infants hypertrophic cardiomyopathy is a known complication of steroid therapy, developing with steroid courses of 2–3 weeks duration or longer[7]. However, hypertrophic cardiomyopathy has not been reported in the context of betamethasone, which is frequently used by local quacks as a part of cough remedies. Myocytes respond by increase in protein synthesis for prolonged use of steroid therapy, leading to hypertrophy of myocardium in premature neonates and young infants. Such changes are transient in premature infants, as they resolve within 1–2 weeks after discontinuation of the steroids[7].. In older children it may take longer for ventricular hypertrophy to resolve, therefore there is need for longer follow-up[8]. Alpert reported HCM in a 14-month-old baby on high-dose steroids for hypsarrhythmia and infantile spasms, with the changes regressing in 1 year on withdrawal of steroids [9]. Septal hypertrophy causing venturi effect which draws the anterior mitral leaflet against the ventricular septum, resulting in outflow obstruction and a pressure gradient develops. The same phenomenon, systolic anterior motion (SAM) of mitral valve was present in our case. SAM can be seen in steroid-induced hypertrophic cardiomyopathy [10].

Balys et al reported a 4-month-old baby who developed HOCM following dexamethasone treatment for subglottic stenosis. The baby had clinical worsening hemodyanamics which improved on stoppage of the medicine[11]. Scire`et al reported marked LVH mimicking HCM in an child treated with steroids for congenital adrenal hyperplasia (CAH), in which the LVH regressed on reducing the drug dosage [12].

CONCLUSION:

Infants receiving glucocorticoids should undergo regular echocardiographic monitoring, with high index of suspicion for hypertrophic cardiomyopathy. Stopping of steroids after diagnosing HCM would be life saving.

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Osteochondrodysplasia: A Heritable Disorder

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Case Report

ABSTRACT

We report a case of a 25 years old pregnant lady in her 2nd pregnancy reported for prenatal Ultrasonography, which revealed an estimated fetal weight of 428 grams alongwith shortening of the long bones, short neck, cloverleaf skull deformity and thoraco abdominal disproportion. The case was diagnosed as Osteochondrodysplasia.

KEY WORDS: achondrogenesis, clover leaf skull, osteogenesis imperfecta Type 2, ostochondrodysplasia, somatic mosaicism, thanatophoric dysplasia

INTRODUCTION:

The skeletal dysplasia also termed as osteochondrodysplasia is a heritable group of disorders which primarily affect bone and cartilage, but can also involve muscle, tendons and ligaments. These are inherited as either autosomal dominant, autosomal recessive or X linked disorders and some result due to imprinting errors, somatic mosaicism, and teratogen exposure during pregnancy. Recent advances in imaging modalities has improved our abilities to recognize osteochondrodysplasia in the prenatal period.[1,2]. Some dysplasias are lethal in perinatal period and detected on antenatal ultrasound scans, while the nonlethal dysplasia present early in infancy or childhood with disproportionate short stature, failure of linear growth or with other physical deformities. The three most common lethal skeletal dysplasias are thanatophoric dysplasia, osteogenesis imperfecta Type 2, and achondrogenesis. Many pregnant women are offered an array of noninvasive tests to determine if their fetuses are at risk for genetic disorders. [3, 4] Recent advances in imaging modalities has improved our abilities to recognize osteochondrodysplasia in the prenatal period.[1,2]

CASE REPORTS:

A 25 years old pregnant women in her 2nd pregnancy with one previous normal delivery was referred for prenatal USG scan in 26th week of pregnancy according to her last menstrual period. Her family history was normal. A prenatal USG biometry revealed an estimated fetal weight of 428 grams along with shortening of the long bones, short neck and cloverleaf skull deformity. A thoraco abdominal disproportion was also evident which suggested a high probability of severe pulmonary hypoplasia. Preterm normal vaginal delivery occurred at 32 weeks and infant died within 30 minutes after birth.

DISCUSSION:

Dysplasias are the conditions associated with bone and/ or cartilage growth or texture.[5] The appropriate diagnosis of skeletal dysplasia is dependent upon the integration of clinical and family history, physical examination, radiological examination and molecular and biochemical tests. Among these, a radiological evaluation is an integral part of the diagnostic workup of a dysplasia. For the early diagnosis of skeletal dysplasia a well organized scan of each and every fetus starting from head to toe should be done. In case of any anomaly, extensive scan along with karyotyping and if required consultation with a pediatric surgeon should be sought. Some dysplasias are lethal in perinatal period whereas others presents in infancy or childhood with disproportionate short stature, failure of linear growth or with other physical deformities. Ultrasound helps to discriminate between lethal and non lethal forms of skeletal dysplasias and if a lethal anomaly is detected termination of pregnancy is considered or can be closely monitored with parents fully counseled about the prognosis. Extensive examination of the parents and family members is needed to predict the risk in the

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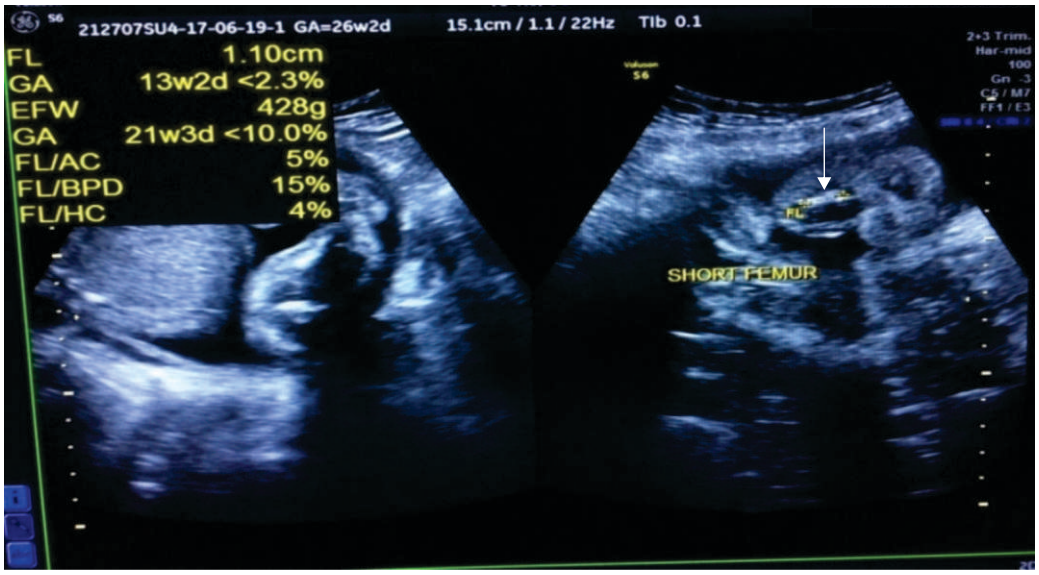

Figure 1: USG image showing short femur.

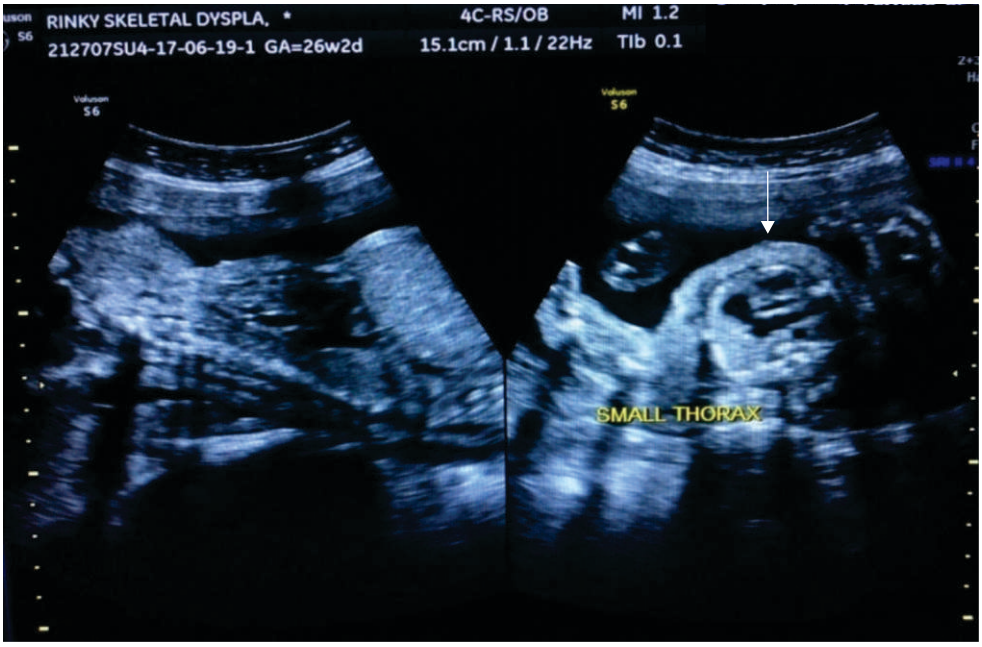

Figure 2: USG image showing small thorax.

next pregnancy. Majority of fetal skeletal malformations can be relatively easily visualised during ultrasound scans and hence ultrasound is the most sensitive way of prenatal diagnosis of these anomalies. However a specific diagnosis depends on the molecular genetics and post mortem examination. The advances in molecular genetics has allowed for gene identification in more than two thirds of the skeletal dysplasias. Chromosomal abnormalities (numerical & structural) are found in most of the patients, of which numerical abnormality (monosomy) is more common as compared to structural abnormalities.

CONCLUSION:

The complete group of osteochondrodysplasia, although individually rare, but it is an important group of disorders which present with significant morbidities due to destruction of bone and cartilage caused by defects in linear growth, bone

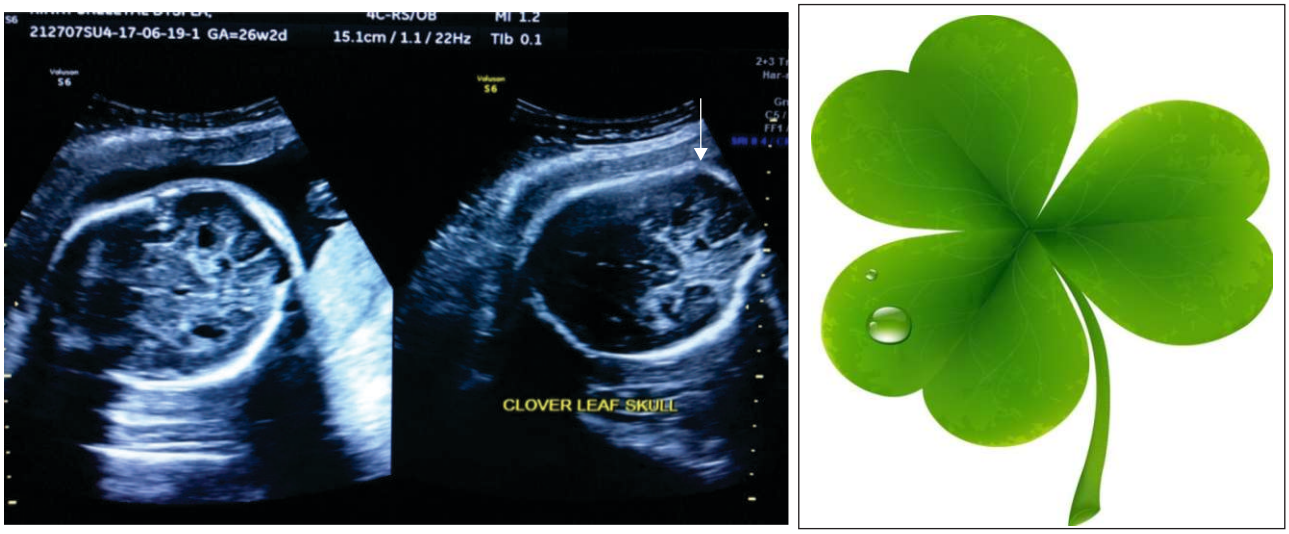

Figure 3: USG image showing clover leaf skull.

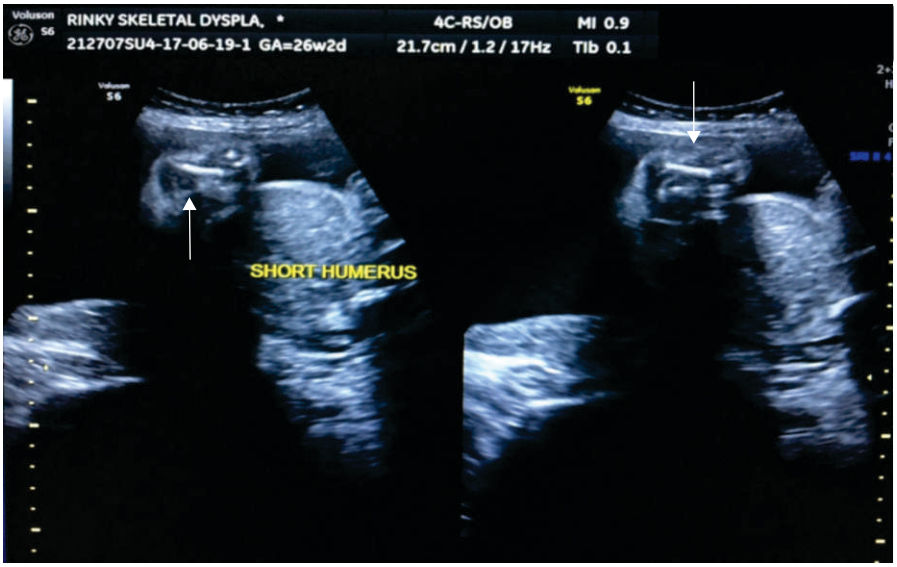

Figure 4: USG image showing short humerus.

modeling and regeneration. Regardless of the specific diagnosis, skeletal dysplasias in general share clinical and radiological findings helping us to group those in several ways. Hence, ultrasound of a fetus suspected to have a skeletal dysplasia is indicated and used to counsel the patient and plan further management.

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